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OFFICIAL ORGAN OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

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and the part CP has played

1822—
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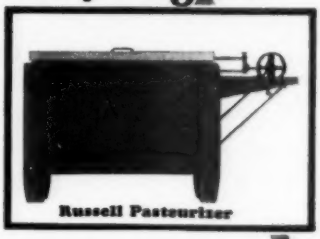
AS a pioneer pasteurizer builder, The Creamery Package Mfg. Company believes it opportune to review briefly some historical facts.

Louis Pasteur, for whom the process is named, was born December 27, 1822 and died September 28, 1895. While he did not work with milk, he laid the foundation upon which the whole structure of modern milk pasteurization is built.



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Milk was pasteurized in a relatively long, deep and narrow vat surrounded by a water jacket. A flat blade agitator moved back and forth in the milk. Another traveling agitator operated in the water space to facilitate exchange of temperature. Heating, holding and cooling, all were accomplished within the machine. Two features worth mention were, the overlapping cover which prevented entrance of dust and moisture, and, the round bore outlet cock located within the water space and operated by handle from above. The apparatus was crude but bacteriologically sound and would pass muster today.

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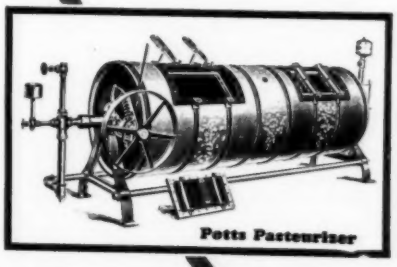

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We show below the effects of applying B-K to the farm utensils, and also applying B-K to the pasteurizing equipment by the spray method without heat.

The results show not only a high reduction of bacterial count in the composite farm milk before pasteurization, but also the thoroughness with which B-K spraying sterilized the factory equipment, thus preventing possible re-contamination.

Immediately before milking time, each farmer used the regular B-K rinse on all buckets, cans and other containers making contact with the milk at the farms, thus accomplishing a reduction in count of the composite farm milk from the high records close to the million mark, previous to using B-K, down to a minimum of 28,000 when tested at factory, the highest single count being 125,000. These counts covered samples from every run through the three weeks.

At the milk plant, the only method used for sterilizing the equipment was spraying with B-K the vats, pasteurizer, cooler, filler, etc., and a flowing B-K solution through pipes, valves, and similar places not accessible for the spray.

The average of all counts in plant for the whole period using B-K as the only means of sterilizing equipment, is as follows:

Condition of composite milk in vat before pasteurization,	*sample No. 1,*	*50,000*
Condition of composite milk, after pasteurization..	*sample No. 2,*	*1,000*
Milk over cooler.....	*sample No. 4,*	*1,000*
Milk from filler.....	*sample No. 5,*	*1,500*
Milk from bottle.....	*sample No. 7,*	*2,000*
Condition of composite milk in vat before pasteurization,	*sample No. 1,*	*28,000*
Condition of composite milk after pasteurization...	*sample No. 2,*	*500*
Milk over cooler.....	*sample No. 4,*	*450*
Milk from filler.....	*sample No. 5,*	*500*
Milk from bottle.....	*sample No. 7,*	*550*

The actual cost of B-K was less than six cents per thousand gallons of milk, and the labor cost of applying it was seven cents per thousand gallons, the total being vastly less than the cost of applying steam.

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CONCERNING THE ADDITION OF CALCIUM CHLORIDE TO MILK FOR CHEESE MAKING*

W. V. PRICE

Department of Dairy Industry, Cornell University, Ithaca, New York

The experiments whose results are given in this report are a continuation of work begun by Georges Knaysi and J. D. Nelson (1). These workers reported an increase in the yield of cheese obtained from a given amount of milk by the addition of $CaCl_2$. They believed that this increase in yield might be due to the retention of more of the milk fat, solids-not-fat, and moisture in the cheese. Their experiments were few in number but pointed out the possibility of producing cheese more economically through the use of $CaCl_2$. They have reviewed the important literature on the subject so that it is necessary here to merely call attention to their report.

The experiments in cheesemaking of the present study have been conducted under carefully controlled conditions which approximate commercial methods of manufacturer accompanied by careful and exact observations and measurements.

The milk used to make the cheese was of varying quality. At least 600 pounds was thoroughly mixed in an ice cream batch mixer after adding starter in amounts sufficient to produce the desired development of acidity during the cheesemaking process. Two portions of exactly 300 pounds each of this mixture of milk and starter were drawn from the mixing tank and placed in two 50-gallon jacketed cheese vats. Each portion was sampled and tested for fat and total solids as a check upon the identity of the milk in each vat.

One-tenth of one per cent or 4.6 ounces of anhydrous $CaCl_2$ was dissolved in 2 pounds of water and added to the test vat of milk. Two pounds of water were added to the check vat to insure the same moisture content in both lots of curd at the time of cutting.

* Received for publication October, 1926

The $CaCl_2$ stimulated the action of the rennet to such an extent that it was necessary to reduce the amount of rennet added to the milk in the test vat to one-half the amount used in the check vat. Even under these conditions the coagulation of the $CaCl_2$ treated milk was more rapid than the coagulation of the milk in the check vat. Further decreases in the amount of rennet were held to be inadvisable due to the probable effect upon the subsequent ripening of the cheese.

The direct result of the influence of the $CaCl_2$ on the rennet action was a faster and firmer coagulation and a more rapid loss of moisture after cutting. At the time of pressing, however, the

TABLE 1

	AVERAGE YIELD FROM 300 POUNDS OF MILK	POUNDS OF FAT	POUNDS OF SOLIDS-NOT-FAT	POUNDS OF MOISTURE
$CaCl_2$	30.388	10.480	9.103	10.800
Check	30.210	10.360	9.015	10.830
Difference	0.178	0.120	0.088	−0.030
Odds that the difference is significant	132 to 1 Significant	267 to 1 Significant	1.1 to 1 Insignificant	3.4 to 1 Insignificant

The average yield and composition of the cheese obtained from 300 pounds of milk treated with 0.1 per cent of $CaCl_2$ and of the cheese made from 300 pounds of identical milk containing no added $CaCl_2$.

moisture contents of the two lots were practically identical. This ability of milk treated with $CaCl_2$ to lose moisture very rapidly during the critical periods of the cheesemaking process has a direct application in the handling of milk with a high acidity at the time of renneting. The shortening of the period in which it is possible to firm the curd sufficiently to insure a sweet cheese allows the cheese maker to remove the whey before the acidity has reached the dangerous limit.

The results of the experiments have been summarized in tables 1 and 2. The average differences in the yield of the cheese and the composition of the cheese and whey have been calculated and the significance of these differences determined by Student's method for interpreting the results of paired experiments (2, 3).

Each 300 pounds of milk treated with $CaCl_2$ produced on the average only 0.178 pound more of cheese than milk not so treated. The real cause of the increase in the yield was obviously the retention of more of the milk fat in the curd. The cheese containing the $CaCl_2$ retained slightly greater amounts of solids-

TABLE 2

	POUNDS OF FAT	POUNDS OF SOLIDS-NOT-FAT
$CaCl_2$	1.017	17.575
Check	1.133	17.502
Difference in pounds	−0.116	0.073
Odds that the difference is significant	9999 to 1	5 to 1
	Significant	Insignificant

The average composition of whey obtained from 300 pounds of milk treated with 0.1 per cent of $CaCl_2$ and of the cheese made from 300 pounds of identical milk containing no added $CaCl_2$.

TABLE 3

Average scores

LOT	FLAVOR		BODY AND TEXTURE	
	$CaCl_2$	Check	$CaCl_2$	Check
1	39.7	38.7	22.5	22.8
2	40.0	40.3	23.2	23.2
3	38.8	38.3	22.5	21.7
4	42.0	43.3	22.8	23.8
5	40.3	40.0	23.5	23.5
6	39.0	39.7	22.8	23.2
7	38.7	39.7	22.8	23.0
8	40.3	42.0	23.3	24.2
9	39.3	39.0	23.2	22.8
10	42.2	44.0	23.2	24.0
Mean	40.03	40.50	22.98	23.22

not-fat than the check cheese but the difference was insignificant. The moisture content of the two types of cheese was practically identical.

The composition of the whey from the two lots of milk also demonstrates the retention of the fat and other milk solids in the cheese. Table 2 shows that the whey from the $CaCl_2$ treated

milk contained 0.116 pound less fat than did the whey from the untreated milk. A slightly greater amount of milk solids not fat in the whey from the $CaCl_2$ treated milk is due probably to chance according to Student's method of interpreting the results of the experiments.

The cheese made in these experiments were scored at eleven months of age. Three judges examined the cheese and their average scores on flavor and body and texture are given in table 3. A score of 50 designates a perfect flavor and 25 a perfect body and texture.

The data of table 3 illustrate the variable quality of the cheese which is due primarily to the quality of the lots of milk from which they were made. Considering the variable nature of the data, the mean scores show little difference in either the flavor or body between the two types of cheese.

The actual scores of the judges were changed to the corresponding ratio scores (4), and these ratio scores were treated by Student's method of determining the significance of the difference of paired observations.

The mean gain in ratio score of the cheese from the untreated over the cheese from the $CaCl_2$ treated milk is 8.18 points. The odds, however, are only 9.4 to 1 that the difference is significant. These odds are too small to state definitely that the $CaCl_2$ milk does not make as good cheese as the non-treated milk.

The mean ratio score of the test cheese is 42.3 ± 3.5 and the cheese from the non-treated milk show an average of 50.4 ± 6.7 points. The small number of observations and the great variation in the quality of the milk from which the cheese were made account for the large probable errors.

The results of the scoring of the cheese do not prove definitely that the $CaCl_2$ has a harmful effect upon the quality of the cheese.

REFERENCES

(1) Knaysi, Georges, and Nelson, J. D.: Jour. Dairy Sci., vol. x, no. 4, p. 396, 1927.
(2) Love, H. H., and Brunson, A. M.: Jour. Amer. Soc. Agronomy, vol. 16, no. 1, January, 1924.
(3) Love, H. H.: Jour. Amer. Soc. Agronomy, vol. 16, no. 1, January, 1924.
(4) Price, W. V.: Jour. Dairy Sci. vol. x, no. 1, pp. 41-44, 1927.

GRAPHICAL STANDARDIZATION OF CONDENSED MILK PRODUCTS*

WALTER V. PRICE

Department of Dairy Industry, Cornell University, Ithaca, New York

The manufacturer of condensed milk products is confronted with a problem of standardizing peculiar to the condensing industry. The finished products are derived from raw materials whose proper combination has established a desired ratio of fat to milk solids not fat. This ratio is usually stated $\frac{\text{Fat}}{\text{Milk solids not fat}}$. The simple adjustment of these ratios, through the mixing of raw materials like cream, milk, skimmilk, etc., which is rather involved by the usual methods of calculation, can be simplified and shortened by the use of a graph.

For the purpose of explanation it is assumed that an ice cream mix is to be made in the condensing pan which must contain 12 per cent fat, 10 per cent milk solids not fat, 14.5 per cent sugar and 0.5 per cent gelatine, and the completed mix must weigh 3000 pounds. The milk products to be used are milk containing 4 per cent fat and 8.8 per cent milk solids not fat, and cream containing 30 per cent fat and 6.3 per cent milk solids not fat.

It is evident that if it is to be completed in the pan, the mix before condensing should contain the desired amount of the solid elements which should bear to each other the ratio required by the finished product. It is obvious that whatever the combination of milk and cream may be, 435 pounds of sugar and 15 pounds of gelatine must be in the mixture. The information desired, therefore, is the pounds of 30 per cent cream and 4 per cent milk which, combined, will make a mixture containing 12 pounds of fat for each 10 pounds of milk solids not fat, with a total of 360 pounds of fat and 300 pounds of milk solids not fat.

In the graph shown in figure 1 the per cent of fat is plotted on

* Received for publication August 1, 1926.

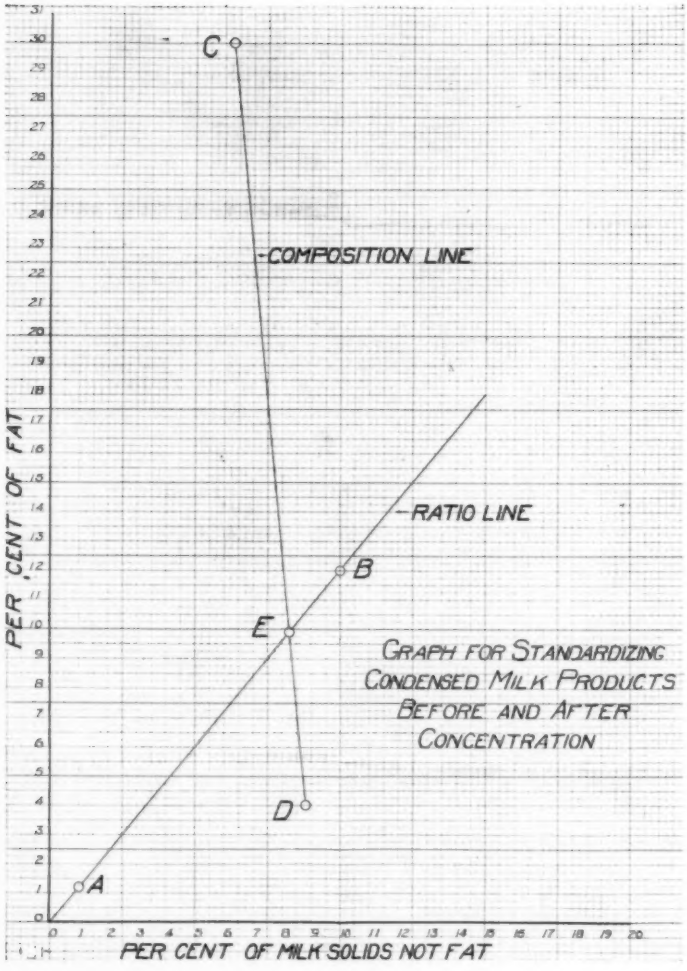


FIG. 1. THE GRAPH ILLUSTRATED WAS DRAWN ON STANDARD, CROSS SECTION, MILLIMETER PAPER TO THE SCALE: 2 CENTIMETERS EQUAL 1 PER CENT

the ordinate and the per cent of milk solids not fat on the abscissa.

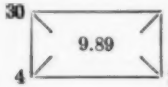
The desired mix must contain fat and milk solids not fat in the ratio of $\frac{360}{300}$ or $\frac{12}{10}$ or $\frac{1.2}{1}$, etc. Therefore, if a theoretical mixture of milk products contained 1.2 per cent of milk fat and 1 per cent of milk solids not fat, it would satisfy the ratio of fat to milk solids not fat required by the problem. This composition is represented on the graph by the point *A*. A theoretical mixture of milk products containing 12 per cent of milk fat and 10 per cent of milk solids not fat would also satisfy the ratio of fat to milk solids not fat required by the problem. Point *B* is plotted on the graph to represent this composition. The line determined by the points *A* and *B* is the locus of all points in which the ratio of fat to milk solids not fat is 12 to 10. It will be called "the ratio line" and is determined by the points representing the composition of any two mixtures which contain fat and milk solids not fat in the desired ratio.

A point indicating the composition of the cream is plotted on the graph at *C*. The milk composition is indicated by a point *D*. The line drawn from *C* to *D* contains the points representing the compositions of all the possible combinations of these samples of milk and cream. At *C* the mixture of the two materials contains 100 parts of cream and 0 parts of milk; at *D* the mixture consists of 100 parts of milk and 0 parts of cream. *CD* may be called "the composition line."

The line *CD* intersects the line *AB* at the point *E*, which is the only point in the composition line *CD* which represents fat and milk solids not fat in the ratio of 12 to 10. Point *E* therefore designates the composition of the mixture of milk and cream which when condensed will make a mix containing 12 per cent of fat and 10 per cent of milk solids not fat. Estimated from the position of point *E* on the graph this composition is 9.89 per cent fat and 8.23 per cent milk solids not fat. Several methods can be used to proportion correctly the amounts of milk and cream necessary to make such a mixture.

Pearson's square may be used to determine the pounds of milk

and cream necessary to make the mixture of milk and cream containing 9.89 per cent fat and 8.23 per cent msnf. The pounds of this mixture necessary to furnish the total number of pounds of fat and solids not fat can then be calculated. The calculations by the square method may be based upon either the per cent of fat or milk solids not fat in the milk products. It is usually desirable, however, to so adjust the materials that any small error due to incorrect reading of the graph will occur in the amount of milk solids not fat in the total mixture. This will be automatically taken care of if the calculations are based upon the fat composition.



5.89 unit parts of 30 per cent cream

20.11 unit parts of 4 per cent milk

26.00 unit parts of 9.89 per cent mixture

Since 3000 pounds of the mix requires 360 pounds of fat, it follows that the pounds of 9.89 per cent mixture necessary to furnish this amount of fat is $\frac{360}{9.89} \times 100$ or 3640.04 pounds of milk and cream mixture.

Since 5.89 parts of cream and 20.11 parts of milk give 26.00 parts of 9.89 per cent mixture, then the amount of cream required to make 3640.04 pounds of mixture is:

$$\frac{5.89}{26.00} \times 3640.04 = 824.61 \text{ pounds}$$

and the amount of milk is:

$$\frac{20.11}{26.00} \times 3640.04 = 2815.43$$

Proof in pounds

	MATERIAL	FAT	MSNF
Milk	2815.4289	112.617	247.757
Cream	824.6088	247.382	51.950
Total	3640.0377	359.999	299.707

No further calculations are necessary. The pounds of sugar and gelatine required may be added to the milk products and the mixture condensed to a total weight of 3000 pounds, when it will have the desired composition.

The proportioning of the amounts of milk and cream can be accomplished in another manner. It has been shown that the line CD represents the composition of the mixtures of milk and cream. Since at point C there are 0 parts of milk in the mixture, and at point D there are 100 parts of milk in the mixture, it follows that points intermediate between C and D indicate increasing parts of milk per 100 parts of mixture as the positions of these intermediate points approach the composition D. From the graph it is evident that point E is nearer D than C, and therefore the desired milk and cream mixture must contain more milk than cream. The exact parts of milk per hundred of mixture would be known if the length of CE relative to the length of CD were known. It can be shown geometrically that the length of the lines CE, ED, and CD are proportional to the vertical distances between the abscissas in which C, E, and D are located. These abscissas on the graph are designated as 30, 9.89, and 4 per cent respectively. It follows that:

$$\frac{CE}{CD} = \frac{30 - 9.89}{30 - 4} \tag{1}$$

and

$$CE = \frac{(30 - 9.89)\ CD}{30 - 4} \tag{2}$$

The length of the line from C to D represents an increase of from 0 to 100 parts of milk in 100 parts of mixture, so that CD may be said to be equivalent to 100. Equation (2) now becomes:

$$CE = \frac{(30 - 9.89)100}{30 - 4} \tag{3}$$

$$CE = 77.346 \tag{4}$$

The value 77.346 represents the parts of milk in 100 parts of the mixture of milk and cream, and 100 less 77.346 equals 22.654, the parts of cream per 100 parts of milk and cream when the fat con-

tent of the milk, cream, and mixture are 4 per cent, 30 per cent and 9.89 per cent respectively.

Since the 3000 pounds of mix required 360 pounds of fat, the pounds of 9.89 per cent mixture necessary to furnish this amount of fat is $\frac{360}{9.89} \times 100$ or 3640.04 pounds of milk and cream. It has been shown that 77.346 per cent of 3640.04 equals the pounds of milk required, and 22.654 per cent of 3640.04 equals the pounds of cream in the mixture. The proof of the method may be shown by making the necessary calculations and arranging the amounts of the products and their fat and milk solids not fat contents in tabular form.

Proof

MATERIAL	POUNDS	POUNDS OF FAT	POUNDS OF MSNF
Milk	2815.42	112.617	247.75
Cream	824.62	247.386	51.95
Total	3640.04	360.003	299.70

No further calculations are necessary. The sugar and gelatine required may be added directly to the milk and cream before or after condensing, as the operator desires. In either case the amount of raw material, which totals 4090 pounds, must be reduced by the removal of water to a total weight of 3000 pounds for the finished product.

The form of graph illustrated has one or two other interesting characteristics and applications. The line *CE* and *CD* might be measured in units of length and the amounts of milk and cream calculated by the proportion

$$\frac{\text{Pounds of milk}}{\text{Pounds of mixture}} = \frac{\text{Length of } CE}{\text{Length of } CD}$$

or

$$\frac{\text{Pounds of cream}}{\text{Pounds of mixture}} = \frac{\text{Length of } ED}{\text{Length of } CD}$$

The graph may be used to standardize off batches of condensed products. No calculations are necessary to determine if the mixture to be standardized is short or over on fat or milk solids not fat. If fat is needed the point representing the composition of the incorrect mixture will fall *below* the ratio line, and if milk solids not fat are needed the point will fall *above* the line. In either case the point representing the composition of the material which can be used to adjust the ratio must fall on the *opposite* side of the ratio line, so that the line connecting the two points will intersect the ratio line. If the point of intersection of the ratio and composition lines indicates a fat and milk solids not fat content in the mixture greater than that desired in the finished product, then water must be added to the milk products. If the point of intersection indicates a fat and milk solids not fat content less than that desired in the finished product, then water must be removed by condensing.

For laboratory work a large graph mounted on a drawing board can be used to good advantage. The ratio lines for the various products manufactured can be drawn on the graph in fine lines and the points representing the composition of the different materials can be marked by glass headed pins which can be inserted where desired and connected by fine black thread in place of drawn lines. This procedure makes it possible to use the graph for a long time without changing the graph paper. A large graph makes possible more accurate standardization.

SUMMARY

1. The ratio line is the locus of all points in which $\frac{\text{Fat}}{\text{Milk solids not fat}}$ equals the ratio desired in the finished product.

2. The line connecting the points representing the compositions of the materials to be combined in the mixture is the locus of all points representing the composition of every possible combination of these materials alone.

3. The intersection of the ratio line and composition line is a point which represents the only combination of the materials used which can furnish fat and solids not fat in the desired ratio.

MOLD AND YEAST COUNTS AND THEIR RELATION TO THE COMPOSITION OF BUTTER*

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The mold and yeast counts of butter have attracted considerable attention during recent years. Lund, Hood, Bouska and others have presented data to show the value of these counts in the control of manufacturing methods. A certain degree of success has been attained in improving the quality of butter through the interpretation of these mold and yeast counts. This improvement has been effected largely through the more efficient pasteurization of the cream and better sanitation in the creamery. Unquestionably, these counts are fair indices of the general sanitation in the creamery. While they cannot be taken per se as criteria of efficient pasteurization, high counts may often be traced to insufficient heating or holding, or both. Routine analyses of butter, including the determination of the mold and yeast counts, make it possible for a supervisory agency to trace difficulties in creamery practice.

Under practical conditions, however, one must always take into account a combination of circumstances, including the time element, temperature and the composition of the butter when the mold and yeast counts are to be interpreted. Bouska points out, for instance, that counts made upon unsalted butter are unreliable after the butter is twenty-four hours old, due to the fact that there is the probability of the growth of the microorganisms particularly if the temperature and other conditions are favorable.

There is a great deal that is still to be learned concerning the factors which influence the development of mold in butter. Thom has demonstrated that the amount of salt present is a

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factor of much importance. However, the information available upon this point is relatively meager and deserves further study. Factors, such as moisture content of the butter, humidity of the atmosphere, temperature, physical and chemical constitution of the butter and others, are challenges for extensive investigation. The utmost value of the mold and yeast counts cannot be reached until we have cleared up some of the unknown variables.

We have under way in our laboratories extensive studies upon the factors influencing the development of mold in butter. In connection with these investigations, we have had occasion to make mold and yeast counts of a large number of samples of butter from several hundred creameries. At the same time, the composition of the butter was ascertained.

The data obtained in this survey serve as a starting point for further studies. The material which is offered at this time is to be considered as preliminary and will be amplified later by controlled experiments.

SOURCE OF MATERIAL

Samples of 2700 lots of butter of varying composition, age and quality were taken at random from 60-pound tubs as they were shipped to the eastern markets.

METHODS OF ANALYSIS

A butter trier was used to remove a plug of butter from the tub. This sample was then placed in a sterile 4-ounce glass jar, provided with an aluminum screw top.

The mold and yeast counts were made after melting the butter at 98° to 100°F. by plating 1 cc. of the melted butter. The plates were poured with whey agar. Before pouring, 1 cc. of 1 per cent sterile tartaric acid solution was added to each plate. The plates were incubated at room temperature (20° to 25°C.) for three days before counting. Counts are reported per cubic centimeter of butter.

The modified Kohman method was used for the determination of the moisture and salt. The curd was arbitrarily assumed as 1 per cent and the fat calculated by difference.

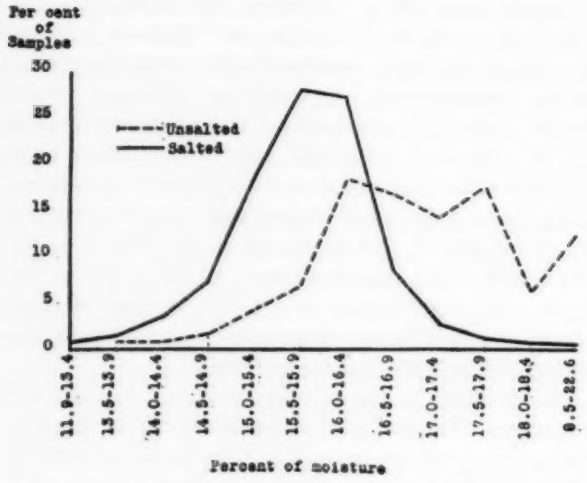

FIG. 1. DISTRIBUTION OF SAMPLES ON THE BASIS OF MOISTURE CONTENT

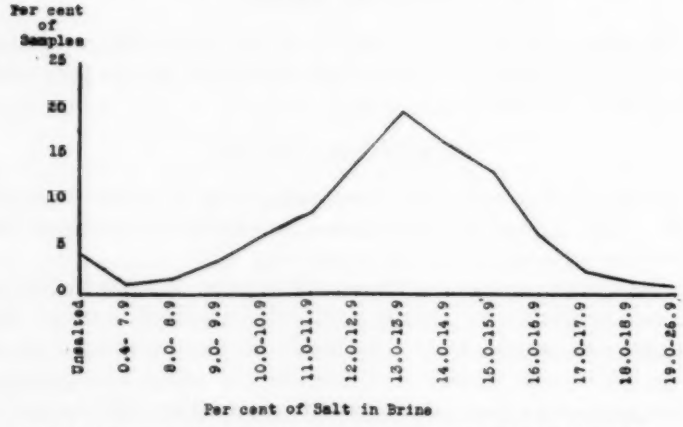

FIG. 2. DISTRIBUTION OF SAMPLES ON THE BASIS OF SALT CONTENT

PRESENTATION OF DATA

The percentage distribution of the samples upon the basis of the percentage of moisture, salt and "salt in brine" is indicated

in figures 1 to 3. These serve as a background for the presentation of the tables which follow.

It will be noted that the samples have been arranged in groups in each chart. Some of the extreme groups represent a consolidation of samples over a wide range because of the small number of samples. Otherwise the groups are within limits of 0.5 or 1 per cent, as the case may be. The distribution charts should be self-explanatory.

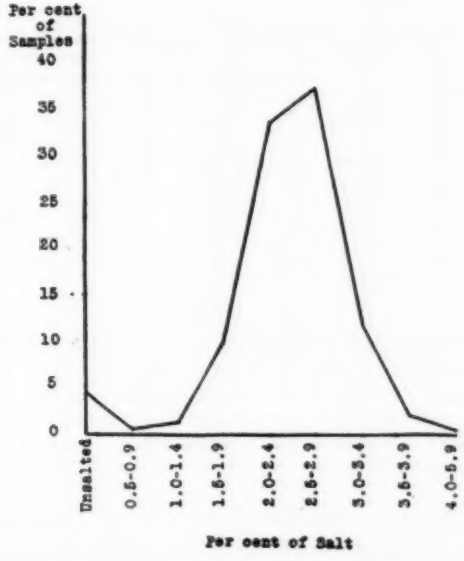

FIG. 3. DISTRIBUTION OF SAMPLES ON THE BASIS OF PERCENTAGE OF SALT IN BRINE

In presenting the mold and yeast counts, individual counts of the samples are not given. It is obvious that it would be impractical to give the figures on each of the 2700 samples. Consequently, the counts on the various samples have been segregated into four classes, namely, those with counts of (*a*) 10 or less, (*b*) 11 to 50, (*c*) 51 to 100 and (*d*) 101 and over.

Counts of 10 or less may be considered as evidence of excellent

work in the creamery while those over 100 are to be rated as poor or at best questionable.

The percentage of samples falling into each class has then been determined for each group of samples previously set up on the basis of the composition of the butter. Thus, it will be noted in table 1 that 13.3 per cent of the samples having a moisture content of 11.9 to 13.4 per cent showed a total count of 10 or less, while 33.4 per cent had a total count between 11 and 50, and so on.

TABLE 1

Relation of total mold and yeast counts to moisture content (salted butter)

MOISTURE CONTENT OF BUTTER	DISTRIBUTION OF TOTAL COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
11.9–13.4	13.3	33.4	13.3	40.0
13.5–13.9	10.0	40.0	12.5	37.5
14.0–14.4	9.5	32.6	13.7	44.2
14.5–14.9	11.6	31.8	23.3	33.3
15.0–15.4	15.9	32.2	23.5	28.4
15.5–15.9	13.5	34.4	23.1	29.0
16.0–16.4	18.3	37.1	21.4	23.2
16.5–16.9	10.2	35.4	23.7	30.7
17.0–17.4	15.1	36.4	10.6	37.9
17.5–17.9	3.7	51.9	29.6	14.8
18.0–18.4	13.3	26.7	40.0	20.0
18.5–22.6	8.3	16.7	8.3	66.7

Table 1 illustrates the distribution of the total counts in relation to the moisture content of the salted butter. It will be noted that there is no marked relationship between the count and the moisture content. The increased amount of the moisture in the butter does not seem to reflect any marked increase in the percentage of high counts or vice versa. There is a slight tendency toward higher counts in the high moisture butter but the small number of samples in these extreme groups may temper the significance of this fact.

Due to the limited number of samples of unsalted butter, the figures for them are not reported. They show the same tendency

as the salted butters, that is, no marked relationship between total counts and moisture.

TABLE 2

Relation of mold counts to moisture content (salted butter)

MOISTURE CONTENT OF BUTTER	DISTRIBUTION OF MOLD COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
11.9–13.4	53.3	26.7	13.4	6.6
13.5–13.9	45.0	37.5	5.0	12.5
14.0–14.4	47.4	34.7	8.4	9.5
14.5–14.9	47.7	33.3	9.0	10.0
15.0–15.4	51.3	34.0	8.3	6.4
15.5–15.9	48.8	38.3	7.4	5.5
16.0–16.4	54.9	36.1	4.6	4.4
16.5–16.9	46.5	42.3	6.5	4.7
17.0–17.4	43.9	45.5	6.1	4.5
17.5–17.9	55.6	29.6	11.1	3.7
18.0–18.4	46.7	33.3	6.7	13.3
18.5–22.6	25.0	50.0	0.0	25.0

TABLE 3

Relation of yeast counts to moisture content (salted butter)

MOISTURE CONTENT OF BUTTER	DISTRIBUTION OF YEAST COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
11.9–13.4	13.3	46.7	20.0	20.0
13.5–13.9	22.0	36.5	19.5	22.0
14.0–14.4	20.8	36.5	11.4	31.3
14.5–14.9	29.3	35.1	16.8	18.8
15.0–15.4	27.3	41.8	13.3	17.6
15.5–15.9	25.9	40.8	14.7	18.6
16.0–16.4	30.9	39.5	14.6	15.0
16.5–16.9	24.8	36.3	17.1	21.8
17.0–17.4	35.4	23.2	13.4	28.0
17.5–17.9	25.5	29.8	21.3	23.4
18.0–18.4	22.7	36.4	13.6	27.3
18.5–22.6	33.3	8.4	33.3	25.0

In table 2 the data for the mold counts indicate the same lack of direct correlation. This is also true for the unsalted samples.

The relation between the yeast counts and the moisture is shown in table 3. There is no evidence that the moisture content influences the yeast counts. This also holds for the unsalted samples.

TABLE 4

Relation of total mold and yeast counts to salt content

SALT CONTENT OF BUTTER	DISTRIBUTION OF TOTAL COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	0.9	2.7	2.7	93.7
0.5–0.9	0.0	16.7	0.0	83.3
1.0–1.4	6.5	6.5	19.3	67.7
1.5–1.9	10.0	24.4	21.5	44.1
2.0–2.4	12.2	31.9	22.7	33.4
2.5–2.9	17.2	37.1	22.8	22.9
3.0–3.4	16.3	45.7	20.8	17.2
3.5–3.9	22.9	43.8	18.7	14.6
4.0–5.9	42.8	28.6	28.6	0.0

TABLE 5

Relation of mold counts to salt content

SALT CONTENT OF BUTTER	DISTRIBUTION OF MOLD COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	7.0	6.1	8.0	78.9
0.5–0.9	16.7	33.3	0.0	50.0
1.0–1.4	41.9	22.6	16.1	19.4
1.5–1.9	44.1	37.0	7.4	11.5
2.0–2.4	47.2	38.5	8.1	6.2
2.5–2.9	53.4	36.8	5.8	4.0
3.0–3.4	55.3	32.3	7.0	5.4
3.5–3.9	56.3	37.5	2.1	4.1
4.0–5.9	66.7	33.3	0.0	0.0

However, when we come to table 4 we find that there is a marked correlation between the total counts and the salt content of the butter. This is particularly striking in the low and high count columns. As the percentage of salt increases, the

percentage of high counts decreases. The intermediate classes do not demonstrate this quite as distinctly as might be expected.

With the mold counts, as shown in table 5 we observe the same tendency for the high counts to be associated with the

TABLE 6

Relation of yeast counts to salt content

SALT CONTENT OF BUTTER	DISTRIBUTION OF YEAST COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	28.1	14.0	16.7	41.2
0.5–0.9	50.0	16.7	16.6	16.7
1.0–1.4	25.8	6.5	16.1	51.6
1.5–1.9	21.1	32.9	17.4	28.6
2.0–2.4	21.7	39.5	18.0	20.8
2.5–2.9	31.5	40.3	12.9	15.3
3.0–3.4	34.8	46.6	10.9	7.7
3.5–3.9	43.8	45.8	6.3	4.1
4.0–5.9	42.9	57.1	0.0	0.0

TABLE 7

Percentages of salt in brine with various combinations of salt and moisture in butter

MOISTURE CONTENT OF BUTTER	SALT IN BRINE WHEN PERCENTAGE OF SALT IN BUTTER IS					
	1	2	3	4	5	6
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
20	4.76	9.09	13.04	16.66	30.00	23.08
19	5.00	9.52	13.64	17.39	20.83	24.00
18	5.26	10.00	14.29	18.18	21.74	25.00
17	5.56	10.53	15.00	19.05	22.73	26.09
16	5.88	11.11	15.79	20.00	23.81	27.27
15	6.25	11.77	16.67	21.05	25.00	28.57
14	6.66	12.50	17.65	22.22	26.32	30.00
13	7.14	13.33	18.75	23.53	27.78	31.58
12	7.69	14.28	20.00	25.00	29.41	33.33

low salt concentrations. As before, the relationship is particularly marked in the classes with low and high counts.

The yeast counts do not demonstrate such a sharp curve, as will be noted in table 6. Here there is a general tendency for

TABLE 8

Relation of total mold and yeast counts to salt content of brine

SALT CONTENT OF BRINE	DISTRIBUTION OF TOTAL COUNTS IN EACH GROUP			
	10 or less	11 to 50	50 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	0.9	2.7	2.7	93.7
0.4– 7.9	4.3	4.4	8.7	82.6
8.0– 8.9	8.9	17.8	24.4	48.9
9.0– 9.9	11.4	24.0	19.8	44.8
10.0–10.9	15.3	27.9	18.2	38.6
11.0–11.9	11.1	31.7	25.9	31.3
12.0–12.9	10.3	33.9	21.8	34.0
13.0–13.9	15.1	31.2	24.2	29.5
14.0–14.9	16.1	36.4	23.2	24.3
15.0–15.9	15.7	44.4	20.2	19.7
16.0–16.9	19.6	41.1	22.6	16.7
17.0–17.9	17.6	45.6	23.5	13.3
18.0–18.9	22.2	38.9	19.5	19.4
19.0–26.9	31.8	36.4	27.3	4.5

TABLE 9

Relation of mold counts to salt content of brine

SALT CONTENT OF BRINE	DISTRIBUTION OF MOLD COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	7.0	6.1	8.0	78.9
0.4– 7.9	30.5	21.7	8.7	39.1
8.0– 8.9	42.2	44.4	6.7	6.7
9.0– 9.9	46.9	33.3	10.4	9.4
10.0–10.9	47.2	34.7	6.8	11.3
11.0–11.9	44.0	42.0	8.2	5.8
12.0–12.9	51.3	36.6	5.8	6.3
13.0–13.9	47.7	33.0	7.9	5.4
14.0–14.9	53.4	35.6	6.4	4.6
15.0–15.9	53.9	35.3	6.0	4.8
16.0–16.9	57.7	34.0	3.6	4.7
17.0–17.9	48.5	38.2	5.9	7.4
18.0–18.9	63.9	16.7	16.7	2.7
19.0–26.9	59.1	36.4	0.0	4.5

the high salt percentages to be related with low yeast counts but there are some noticeable irregularities. Yeasts do not appear to be quite as sensitive to salt concentration as the molds.

After these relationships were studied it was thought advisable to investigate the correlation between the concentration of the salt in the moisture and the counts. Consequently, the percentage of "salt in brine" was calculated by dividing the percentage of salt (as determined by analysis) in each sample by the sum of the percentage of salt and moisture.

TABLE 10

Relation of yeast counts to salt content of brine

SALT CONTENT OF BRINE	DISTRIBUTION OF YEAST COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	28.1	14.0	16.7	41.2
0.4– 7.9	34.8	8.7	8.7	47.8
8.0– 8.9	24.4	17.8	26.6	31.2
9.0– 9.9	19.8	33.3	13.6	33.3
10.0–10.9	27.3	33.5	15.3	23.9
11.0–11.9	22.6	41.2	17.7	18.5
12.0–12.9	18.7	40.0	19.6	21.7
13.0–13.9	26.6	38.7	15.5	19.2
14.0–14.9	30.2	40.4	14.2	15.2
15.0–15.9	33.6	42.5	11.4	12.5
16.0–16.9	33.9	45.3	10.7	10.1
17.0–17.9	41.2	48.5	7.4	2.9
18.0–18.9	47.2	41.7	5.6	5.5
19.0–26.9	36.4	63.6	0.0	0.0

Table 7 is introduced to illustrate how the percentages of salt in brine would vary with different combinations of salt and moisture. This may assist in the interpretation of the remaining tables. It is recognized, of course, that not all of the salt will be uniformly distributed in the water, a fact which will be mentioned later, but the possible relationships of brine and counts are suggestive.

In table 8, the relation between the total counts and the percentage of salt in brine is illustrated. While the effect is not

as regular as that of salt, it is quite marked, in the classes of low and high counts and particularly the latter. With the increase in the concentration of salt in brine, there is a corresponding decrease in the number of samples with high counts.

When we consider the mold counts in table 9, we find that the effect of the salt concentration in the brine is again marked, although it is not as distinct an effect as that shown by the salt alone. However, it is significant. Here again high mold counts are correlated with low salt concentration.

As we might predict, table 10 shows that the yeasts are not as sensitive to the salt, although there is a somewhat general trend toward higher counts with low percentages of salt in brine.

DISCUSSION OF RESULTS

These samples of typical market butter, varying in age from about one day to two weeks were analysed without regard for that fact. The chemical analyses paralleled the making of the mold and yeast counts. The results simply indicate the possible relationship between the composition of the butter and the mold and yeast counts. They are at least suggestive. There has been very meager information in the literature to show such relationships, especially on a large number of samples of butter. However, it is clearly understood that the results must be interpreted with judgment. We do not know how much effect the various constituents of butter may actually have had upon the growth of the molds or yeasts from the time the butter was made until it was analysed. At any rate, some very good clues have been uncovered. Investigations to elucidate causal factors are now in progress in our laboratory. The results should throw some light on the subject and clear up some of the puzzling details.

There is also a factor to be considered in the interpretation of mold and yeast counts, such as those reported, namely, individual workmanship in a creamery. The operator who is careless about the control of the composition of the butter may be likewise careless about sanitation and pasteurization. This may have influenced discrepancies in the extreme groups.

The most striking feature of the data presented is to be found in the correlation between the salt content of the butter and the mold and yeast counts. The effect of the concentration of salt in brine appears to be significant.

It is well to bear in mind, however, when we are considering the relationship of moisture, salt or brine, that, while the ordinary butter analysis may show, gravimetrically, the total amount of salt or moisture in the entire sample of butter, it does not reveal the nature of the dispersion, the amount of dissolved or undissolved salt, or whether the particles of salt or water, and the combinations of the two are evenly or unevenly distributed throughout the sample. Many of the secrets of the defects in the keeping quality of butter may be found to be hidden in the physico-chemical makeup of butter and the relative distribution of the particles of water, salt, curd and air. The solution of at least a part of the problem of the factors influencing the growth of mold in butter may be found in these delicate balances.

SUMMARY

1. Data are presented for 2700 samples of market butter, setting forth the mold and yeast counts and the composition.

2. The mold and yeast counts are compared with the concentrations of moisture, salt and "salt in brine."

3. Apparently, the moisture content of the butter had no particular relationship to the counts.

4. The mold and yeast counts appeared to be effected to a marked degree by the salt content of the butter.

5. The percentage of "salt in brine" seemed to show an effect upon the mold and yeast counts.

6. The influence of higher concentrations of salt and "salt in brine," appeared more marked for mold than for yeast counts.

INCREASING THE YIELD OF CHEESE BY THE ADDITION OF CALCIUM CHLORIDE TO MILK*

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As a result of his work on the chemistry of milk, Lindet pointed out, in 1913, the possibility of increasing the yield in cheese, from a given amount of milk, by the addition to the milk of $CaCl_2$. A few trials were made by some French cheesemakers, and an increase varying from 1.50 to 7.22 per cent was reported. The experiments reported, however, were not conducted on a scientific basis, and no attempt was made to determine the source of that increase, which might have been due to an increase in the moisture content of the cheese, to a decrease of the fat losses in the whey, to a more complete precipitation of the other milk solids, or to a combination of some or all of the above factors.

The following experiments are preliminary to more extensive ones which will be carried on in this department in order to obtain more complete and accurate data in regard to the magnitude of that increase, its source, and the effect of $CaCl_2$ on the flavor, character, and ripening qualities of American cheddar cheese.

Table 1 sums up the results of four experiments which have been conducted with American cheddar cheese. The milk was mixed in a vat, and two equal quantities weighed out and subsequently treated alike as much as possible, except that one portion received $CaCl_2$ and a smaller amount of rennet extract, so that both portions coagulated nearly at the same rate. The cheeses were weighed as soon as taken out of the press and sampled, and the samples were tested for fat and total solids.

Table 1 shows that a certain increase in cheese was always secured when $CaCl_2$ was added to the milk previous to the addition of rennet extract. The increase probably varies with the

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TABLE 1

Magnitude and source of the increase in cheese resulting from the addition of $CaCl_2$ to the milk

NUMBER OF EXPERIMENTS	WEIGHT OF MILK	TREATMENT	YIELD OF CHEESE	INCREASE IN YIELD DUE TO $CaCl_2$		COMPOSITION OF THE CHEESE	
						Fat	Total solids
	pounds		*pounds*	*pounds*	*per cent*	*per cent*	*per cent*
I	281.0	3.4 ounces dry $CaCl_2$	31.4	1.5	5.0	32.0	59.52
	281.0	No $CaCl_2$	29.9			32.0	59.4
II	283.0	3.4 ounces dry $CaCl_2$	33.7	1.9	6.0	33.5	63.15
	283.0	No $CaCl_2$	31.8			33.5	63.64
III	282.5	4.4 ounces crystallized $CaCl_2$	28.7	1.0	3.6	32.5	62.64
	282.5	No $CaCl_2$	27.7			31.0	63.02
IV		4.4 ounces crystallized $CaCl_2$	26.06	0.88	3.5	30.5	62.61
		No $CaCl_2$	25.18			31.5	62.90

TABLE 2*

Sources of increase in yield of cheese of $CaCl_2$ treated milk

POUNDS OF CHEESE FROM THE SAME QUANTITY OF MILK		POUNDS OF FAT IN THE CHEESE		POUNDS OF S.N.F. IN THE CHEESE		POUNDS OF MOISTURE IN THE CHEESE	
$CaCl_2$	None	$CaCl_2$	None	$CaCl_2$	None	$CaCl_2$	None
31.4	29.9	10.048	9.568	8.641	8.193	12.710	12.129
33.7	31.8	11.289	10.653	9.992	9.584	12.418	11.562
28.7	27.7	9.327	8.587	8.650	8.869	10.722	10.243
26.06	25.18	7.948	7.932	8.368	7.906	9.743	9.342
Totals							
119.86	114.58	38.612	36.740	35.651	34.552	45.593	43.286
Gain of $CaCl_2$ in yield and content of fat, S.N.F., and moisture of $CaCl_2$ over none							
+5.28		+1.872		+1.099		+2.307	

Total gain of each ingredient = 5.278.

Total gain of cheese = 5.28.

* We owe this table to the courtesy of Dr. W. V. Price of this department. It is based on the data given in table 1.

composition of the milk, and also with the amount of $CaCl_2$ added. In experiments III and IV, crystallized $CaCl_2$ was added, and probably that was mainly responsible for the drop in the percentage of increase. One-tenth of one per cent of anhydrous $CaCl_2$ should be added.

In table 2 the sources of that increase are shown to be due to more moisture and fat incorporated, and to an increase in the milk-solids-not-fat retained in the cheese. The firmness of the curd from the milk to which $CaCl_2$ has been added might have cut down the amount of small particles carried away in the whey, but other work that we have done shows that there is a more complete precipitation of the soluble milk solids and seems to confirm Lindet's finding that the addition of $CaCl_2$ to milk results in the precipitation of 4.2 to 15.1 per cent of the soluble milk proteins, but we did not check up his other report that at the same time 26.0 to 40.9 per cent of the soluble milk phosphorus is precipitated.

While the data on hand is still scanty, it shows, nevertheless, remarkable economic possibilities. Not only do we get about 5 per cent more cheese from a given amount of milk, but there is a saving of rennet extract, and we see also possibilities in reducing the amount of starter needed. The curd is more easy to handle and the cheese probably has a superior nutritive value due to a more complete precipitation of the phosphorus and the calcium, while there are yet no indications that either the flavor or the ripening qualities of the cheese are impaired.

The chemical changes involved may be very complex, but it seems probable that, due to the condition of dynamic equilibrium in which the soluble salts of the milk serum probably are, the addition of $CaCl_2$ to milk favors the formation of the insoluble calcium phosphates and citrates at the expense of the phosphates and the citrates of sodium and potassium in which the caseins of the milk are soluble, as shown by Lindet. That, and the depressing effect of the $CaCl_2$ upon the dissociation of calcium caseinate with which it has a common ion, probably explain the more complete precipitation which takes place on the addition of $CaCl_2$ to milk.

We feel thus justified in advocating a thorough trial of the use of $CaCl_2$ in cheese making, and we hope that more data will be forthcoming soon in regard to the points touched on in this paper.

REFERENCES

CAMPS: L'emploi du chlorure de calcium dans la fromagerie au Danemark. Le Lait, 1924, iv, 895–897.

KNAYSI, GEORGES: Some factors other than bacteria that influence the body of artificial buttermilk. Jour. Agr. Res., 1927, xxxiv, 771–784.

LINDET, L.: Solubilité des albuminoides du lait dans les éléments du sérum; rétrogradation de leur solubilité sous l'influence du chlorure de calcium. Bull. Soc. Chim. de France, 1913, 4, xiii, 929–935.

LINDET, L.: Emploi du chlorure de calcium en fromagerie. Le Lait, 1924, iv, 2–7.

MILROY, T. H.: The reaction and calcium content of milk as factors in the coagulation process. Biochem. Jour., 1915, ix, 215–228.

VAILLANT, E.: L'emploi du chlorure de calcium dans la fabrication du fromage de Maroilles. Le Lait, 1924, iv, 7–10.

VAN DAM, W.: Recherches sur la coagulation du lait au moyen de la présure. Rev. Gen. du Lait, 1908–1909, vii, 121–129, 145–155, 169–178.

THE NON-PROTEIN NITROGEN IN CERTAIN DAIRY RATIONS AND THE PARTITION OF NITROGEN IN THE URINE PRODUCED THEREON*

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In a recent publication from this laboratory by Maynard, Miller and Krauss (1) there are reported some nitrogen balance studies comparing the protein efficiencies for milk production of a clover ration and a timothy ration. In these studies digestible crude protein was the basis used for computing the experimental rations and also for comparing certain of the results obtained.

It was recognized that the rations being compared contained a large proportion of their nitrogen in compounds other than proteins and that, since in one ration a much larger proportion of the nitrogen was supplied by roughage than was the case in the other, the rations presumably differed markedly in the forms of nitrogen making up what was considered as digestible crude protein. In view of the difference of opinion as to the usefulness of non-protein nitrogen in animal nutrition and the adoption by certain teachers of the Armsby feeding standard which considers non-protein nitrogenous compounds of no value except as a source of energy, it seemed worth while to embrace the opportunity provided by the nitrogen-balance experiment to study the relative amounts of protein and non-protein nitrogen in the rations, and the partition of this ingested nitrogen in the urine and feces. It was believed that such a study would furnish further data on the question as to whether digestible true protein or digestible crude protein is the better basis for computing protein requirements, and that it would also aid in the interpretation of the

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results of the nitrogen-balance experiment. This paper presents the results of an attempt to study these questions.

REVIEW OF LITERATURE

In 1911, Armsby (2) summarized the evidence regarding the nutritive value of non-protein nitrogen from which he concluded that this nitrogen is much inferior to protein nitrogen for production feeding. In 1917, Armsby (3) again discussed the question, stating that digestible true protein seems a safer basis for the computation of rations. On the other hand the reasons why the amids should not be ignored have been stated by Henry and Morrison (4).

In 1924, Forbes (5) reviewed the question of crude and true protein, deciding that the distinction had largely lost its significance in view of the newer knowledge of protein katabolism and that the use of the crude protein standard should be continued. More recently Mitchell (6) has also expressed a similar view.

Actual experiments regarding the replacement value of non-protein for protein had been carried out principally by German investigators. The idea was early advanced that microörganisms in the digestive tract of ruminants may convert non-protein into protein and the early work along this line has been reviewed by Armsby (2). Within the past few years a number of German investigations have been published which it seems worth while to review because of their contribution to the question of crude and true protein.

Völtz (7), using a full-grown wether, found that a ration in which slightly more than half of the nitrogen was furnished by urea allowed 1.2 grams of nitrogen daily for meat production. From previous trials, it was computed that the use of urea had increased the digestible nitrogen 77.7 per cent.

On a ration in which the only source of nitrogen was urea, the same investigator increased the weight of a wether lamb in nine months from 29.29 kgm. to 41.2 kgm., or 40.8 per cent of the initial weight. The explanation given for this is that the urea was built up into bacterial protein in the digestive tract, and 80 to 90 per cent of this bacterial protein was absorbed. It was calculated that 350 grams of urea would replace 1000 grams of protein. Völtz considers as unproved the view that the amids are worthless as a food for ruminants because they are alleged to have no worth in a production ration, and maintains that the nitrogen content of a ration be measured in terms of digestible crude protein rather than digestible true protein. Salkowski (8) objected to

Völtz's interpretation on the ground that the necessary sulfur for protein formation could not have been furnished by the hydrolyzed straw used in the rations.

Morgen and coworkers (9), experimenting with lactating sheep and goats over a period of several years, found that when 30 to 40 per cent of the protein of a ration suitable for milk production was replaced with urea no appreciable change occurred in the milk production. In the following year, Morgen, Windheuser and Ohlmer (10) pointed out that too much urea was unsuitable.

Following up his earlier investigations with an extensive series of studies on dairy cows, Völtz and coworkers (11) were able to furnish additional proof of the replacement value of urea for milk production. As a result of these trials, the authors recommend supplementing protein-poor rations for cattle and sheep with urea, about 150 grams per day being considered the optimum amount.

Similar results were obtained by Honcamp, Koudela and Müller (12) who found that cows will handle from 150 to 200 grams of urea per head per day without ill effects of any kind, and that on a protein-poor, but easily digestible carbohydrate-rich ration a supplement of urea causes an increase in the amount of milk and fat produced.

Morgen, Windheuser and Ohlmer (13) did not obtain an increase in milk production in sheep and goats by adding urea to low protein rations, but did in almost every case obtain an increase in the amount of fat produced.

Further evidence of the truth of the protein-bacteria theory was obtained by Honcamp and Schneller (14), working with wethers, who found that urea, when added to a ration consisting of roughages, was almost quantitatively excreted in the urine. When easily digestible, carbohydrate-rich feeds were given, a marked positive nitrogen balance resulted. On such a ration, in addition to the activity of the bacteria, a protein sparing action is brought about by the combination of ammonia from urea with organic acids from the carbohydrates.

More light is thrown upon the bacterial activity occurring in the first stomach of ruminants by Schwarz (15). In 100 kgm. of paunch contents he found 2.79 kgm. of microörganisms containing 41 grams of nitrogen or 256 grams of protein, 80 to 90 per cent of which, according to Völtz, could be utilized. It was suggested as being possible that a great part of the protein requirement of ruminants is satisfied indirectly by microörganism-protein into which the plant nitrogen is converted in the paunch. It is also possible that specific organisms break down

the food while others assimilate it. Medium hay contains about 3 per cent digestible protein whereas the microörganisms in the paunch contained 7.4 to 8.3 per cent digestible protein. The author believes that unquestionably through such a concentration of the digestible protein in the paunch its further usefulness in the intestine is made possible.

Based upon the results of the investigations just cited, it would seem that in ruminants the change of non-protein to protein by bacteria may be a considerable factor, and to the extent that this bacterial synthesis occurs the question of quality of protein may be of little importance in ruminants.

EXPERIMENTAL PROCEDURE

The nitrogen-balance experiment, in connection with which the study here described was made, has been reported by Maynard, Miller and Krauss (1) and the reader is referred to this publication for a detailed account of the experimental procedure which furnished the materials for the study here reported.

The plan for the nitrogen-balance experiment provided that three cows were to receive in successive periods a ration of clover hay, silage and grain, and a ration of timothy hay, silage and grain, each ration to supply equivalent amounts of digestible crude protein and total digestible nutrients in accordance with the weight and production of each cow. It was stipulated that the intake of total digestible nutrients should be adequate according to the Morrison standard, but that the protein intake should be limited to an amount that would throw the animals into negative nitrogen balance, under which conditions a comparison of the relative efficiency of the nitrogen of the two rations would become possible.

The studies described in the present paper were made upon the samples of feeds, feces and urine obtained in the nitrogen-balance studies made in two successive years. The first year's study, comprising trial 1 in which clover hay was used, and trial 2 in which timothy hay was used, was carried out in the winter of 1923–1924. The second year's study, comprising trial 3, timothy, and trial 4, clover, was carried out during the corresponding period of 1924–1925.

The feeds and rations

The ingredients of the grain mixtures and the average daily rations consumed are shown in table 1. In trial 1, 35 grams of salt per day were fed; in trial 2, 45 grams, and in trials 3 and 4, 60 grams.

Substantially the same amounts of hay and silage were used in all trials,[1] the grain being varied in kind and amount to supply the additional digestible crude protein and total digestible nutrients necessary to meet the requirements with the roughages in question. Thus, with clover hay and silage, the grain supplied a much smaller percentage of the protein in the ration than where timothy and silage were used as the roughages.

TABLE 1

Ingredients of grain mixtures and average daily rations consumed

TRIAL	COW	COMPOSITION OF GRAIN MIXTURE				TOTAL GRAIN	TIMOTHY HAY	CLOVER HAY	SILAGE
		Corn meal	Ground oats	Wheat bran	Oil meal				
		per cent	*per cent*	*per cent*	*per cent*	*grams*	*grams*	*grams*	*grams*
1	1	59.67	20.17	13.44	6.72	8,358		5,444	18,144
	2	45.19	27.41	18.27	9.13	6,276		5,226	18,144
	3	54.65	22.67	15.12	7.56	10,134		5,225	17,517
2	1	35.29	32.36	21.57	10.78	8,970	5,444		18,144
	2	25.19	37.40	24.94	12.47	6,794	4,536		12,096
	3	26.79	36.61	24.40	12.20	10,722	5,444		18,050
3	4	33.42	33.29	22.19	11.10	8,934	5,460		18,144
	5	35.22	32.39	21.59	10.80	7,146	5,460		18,144
	6	40.79	29.61	19.73	9.87	7,244	4,527		17,085
4	4	36.89	63.81			8,544		5,460	18,144
	5	61.20	38.80			7,224		4,540	18,144
	6	65.47	34.53			6,438		4,540	16,689

The grain mixture for every trial, except number 4, consisted of corn meal, ground oats, wheat bran and linseed oil meal. In trial 4 it was necessary to limit the grain mixture to the two

[1] The exceptions to this statement noted in the table are explained in the publication by Maynard, Miller and Krauss (1).

concentrates lowest in protein to provide the desired plane of protein intake. The clover used in trial 1 was graded on the federal basis as number 2 heavy clover mixed; that used in trial 4 was graded as number 1 clover. The timothy hay used in trial 2 was graded number 2 timothy; that used in trial 3 was graded number 1 timothy. The silage used consisted for the most part of corn which was rather immature and contained few ears.

Distilled water was fed during trials 1 and 2, but during trials 3 and 4 tap water was given. From the results of a recent analysis carried out by Dr. F. R. Georgia of the Department of Chemistry, of the water supply used in trials 3 and 4, it was found that the amount of nitrogen in the water was negligible.

Chemical methods[2]

The feeds and feces were analyzed for total and non-protein nitrogen. The Kjeldahl-Gunning method was used for the total nitrogen determination. Non-protein nitrogen was obtained by analyzing for true protein nitrogen by the albuminoid nitrogen method adopted by the Association of Official Agricultural Chemists and subtracting this result from the total nitrogen.

It was recognized, in view of the recent work on the distribution of nitrogen in proteins and in plant tissues, that this method might have limitations as an accurate measure of the non-protein nitrogen. However, it is the method that has been employed wherever distinctions have been drawn between crude and true protein, and it is upon data obtained by this method that the protein requirement of the Armsby and Kellner feeding standards are based, on the usefulness of which the present studies were expected to throw some light. Both because of these facts and because no other generally recognized method of greater value was available, the method here used was chosen.

For trials 1 and 2, urea nitrogen was determined by the Folin and Youngburg method (16) after extracting the ammonia with permutit. No analysis for ammonia nitrogen was made on the

[2] Except for the total nitrogen determination, all urine analyses were made in the biochemistry laboratory of the Cornell Medical College.

urine collected in trials 1 and 2. In trials 3 and 4 special urine samples were acidified with sulphuric acid for the ammonia determination. Upon analyzing these acidified samples for ammonia by Folin and Bell's permutit method (17) no appreciable amount of ammonia could be detected. Likewise, when analyzed for urea by Folin and Youngburg's method (16) and by the new Sumner method, no appreciable amount of ammonia was found. Thinking that the acidity might be the cause of this, the same methods for determining ammonia and urea were applied to unacidified samples of the same urine, but similar results were obtained. The aeration method of Folin for ammonia as described by Hawk (18) and the aeration method of Van Slyke and Cullen (19) for urea were then tried and reasonable amounts of ammonia and urea were found. The failure of the other methods might have been due to the presence of some compound, possibly one containing an amino group, which prevented the action of Nessler's solution.

Hippuric acid nitrogen was determined throughout by the method of Folin as modified by Kingsbury and Swanson (20).

Creatinine and creatine nitrogen were not determined in any of the trials reported here. However, in order to help explain the large amount of undetermined nitrogen in the urine, these two constituents were analyzed for in the urine of a cow used in a later trial of the nitrogen-balance experiment not included in this paper. Folin's microchemical method (21) was used for both creatinine and creatine.

RESULTS

The distribution of non-protein nitrogen in the feed is given in table 2. It will be seen from a study of this table that the timothy hay used in both cases had a greater percentage of its total nitrogen in the form of non-protein nitrogen than did the clover hay, the figures being, in per cent of total nitrogen, 16.12 and 19.78 for timothy hay, and 2.34 and 10.38 for clover hay. This is in agreement with the results obtained by Grindley and Eckstein (22)[3] who found 15.43 per cent of the total nitrogen of timothy

[3] Colloidal ferric hydroxide used as precipitant.

TABLE 2

Nitrogen content of feeds and feces

COW	FEED	FED	TOTAL N	TOTAL N	NON-PROTEIN N	NON-PROTEIN N	TOTAL N AS NON-PROTEIN N	TOTAL N IN RATION AS NON-PROTEIN N IN ROUGHAGE	NON-PROTEIN N OF RATION FURNISHED BY EACH FEED	NON-PROTEIN N IN FECES
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
				Trial 1 (clover)						
		grams	*per cent*	*grams*	*per cent*	*grams*	*per cent*	*per cent*	*per cent*	*grams*
1	Hay	5,444	1.864	101.48	0.043	2.34	2.31		7.01	
	Silage	18,144	0.348	63.14	0.114	20.68	32.76	7.058	61.95	24.561
	Grain	8,358	1.934	161.64	0.124	10.36	6.41		31.04	
	Ration	31,946	1.021	326.26	0.105	33.38	10.23			
2	Hay	5,226	1.864	97.41	0.043	2.25	2.31	7.970	5.57	
	Silage	18,144	0.348	63.14	0.114	20.68	32.76		51.23	11.573
	Grain	6,276	2.026	127.15	0.278	17.45	13.72		43.21	
	Ration	29,646	0.970	287.71	0.136	40.38	14.03			
3	Hay	5,225	1.864	97.39	0.043	2.25	2.31		4.23	
	Silage	17,517	0.348	60.96	0.114	19.97	32.76	6.107	37.59	15.916
	Grain	10,134	2.027	205.42	0.305	30.91	15.05		58.18	
	Ration	32,876	1.106	363.77	0.162	53.13	14.60			
				Trial 2 (timothy)						
1	Hay	5,444	0.825	44.91	0.133	7.24	16.12		13.73	
	Silage	18,144	0.390	70.76	0.141	25.58	36.15	10.391	48.51	21.747
	Grain	8,970	2.232	200.21	0.222	19.91	9.95		37.76	
	Ration	32,558	0.970	315.88	0.162	52.73	16.70			
2	Hay	4,536	0.825	37.42	0.133	6.03	16.12		16.27	
	Silage	12,096	0.390	47.17	0.141	17.06	36.15	9.687	45.99	5.237
	Grain	6,794	2.263	153.75	0.206	14.00	9.10		37.74	
	Ration	23,426	1.017	238.34	0.158	37.09	15.56			
3	Hay	5,444	0.825	44.91	0.133	7.24	16.12		9.27	
	Silage	18,050	0.390	70.40	0.141	25.45	36.15	8.890	32.57	13.001
	Grain	10,722	2.354	252.40	0.424	45.46	18.01		58.17	
	Ration	34,216	1.075	367.71	0.228	78.15	21.25			

TABLE 2—*Continued*

COW	FEED	FED	TOTAL N	TOTAL N	NON-PROTEIN N	NON-PROTEIN N	TOTAL N AS NON-PROTEIN N	TOTAL N IN RATION AS NON-PROTEIN N IN ROUGHAGE	NON-PROTEIN N OF RATION FURNISHED BY EACH FEED	NON-PROTEIN N IN FECES
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Trial 3 (timothy)										
		grams	*per cent*	*grams*	*per cent*	*grams*	*per cent*	*per cent*	*per cent*	*grams*
4	Hay	5,460	0.723	39.48	0.143	7.81	19.78		16.01	
	Silage	18,144	0.292	52.98	0.144	26.13	49.31	12.929	53.58	16.52
	Grain	8,934	1.903	170.01	0.166	14.83	8.72		30.41	
	Ration	32,538	0.807	262.47	0.150	48.77	18.58			
5	Hay	5,460	0.723	39.48	0.143	7.81	19.78		15.52	
	Silage	18,144	0.292	52.98	0.144	26.13	49.31	14.413	51.94	8.96
	Grain	7,146	2.001	142.99	0.229	16.36	11.44		32.53	
	Ration	30,750	0.766	235.45	0.164	50.30	21.36			
6	Hay	4,527	0.723	32.73	0.143	6.47	19.78		15.33	
	Silage	17,085	0.292	49.89	0.144	24.60	49.31	13.872	58.25	4.69
	Grain	7,244	1.952	141.40	0.154	11.16	7.89		26.42	
	Ration	28,856	0.776	224.02	0.146	42.23	18.85			
Trial 4 (clover)										
4	Hay	5,460	1.995	108.93	0.207	11.30	10.38		32.21	
	Silage	18,144	0.237	43.00	0.084	15.24	35.44	9.868	43.44	12.92
	Grain	8,544	1.370	117.05	0.100	8.54	7.30		24.35	
	Ration	32,148	0.837	268.98	0.109	35.08	13.05			
5	Hay	4,540	1.995	90.57	0.207	9.40	10.38		20.75	
	Silage	18,144	0.237	43.00	0.084	15.24	35.44	10.188	33.65	14.00
	Grain	7,224	1.499	108.29	0.286	20.66	19.08		45.61	
	Ration	29,908	0.809	241.86	0.151	45.30	18.73			
6	Hay	4,540	1.995	90.57	0.207	9.40	10.38		26.32	
	Silage	16,689	0.237	39.55	0.084	14.02	35.44	10.604	39.25	14.70
	Grain	6,438	1.409	90.71	0.191	12.30	13.56		34.43	
	Ration	27,667	0.798	220.83	0.129	35.72	16.17			

hay and 14.22 per cent of that of clover hay in non-protein form. Undoubtedly the stage of maturity is an important factor influencing the non-protein nitrogen content of hay. However, the small figure for clover hay in trial 1 seems unexplainable even on this basis.

The non-protein nitrogen content of corn silage, as shown in table 2, is exceedingly large. Annett and Russell (23) found 43.72 per cent of the total nitrogen of corn silage as non-protein nitrogen. They also showed that in the silo there was a loss of all nitrogenous constituents except non-protein, which gained 83 per cent. Shaw, Wright and Deysher (24)[4] found 61.99 and 61.41 per cent of the total nitrogen of corn silage as non-protein nitrogen. They also demonstrated strikingly that there was a great loss of albuminoid nitrogen in ensiling and a tremendous gain in non-protein nitrogen. That the stage of maturity greatly affected the non-protein nitrogen content of silage was clearly shown by Woodman and Amos (25).[5] Fifty per cent of the true protein was hydrolyzed in the case of very immature silage, while only 23 per cent was hydrolyzed in the case of fairly mature silage. These workers concluded that "the conditions of immaturity are favorable to the extensive splitting up of the protein of the crop into amino acids." The fairly large amount of non-protein nitrogen found in the silage used in the trials reported here is thus in accordance with previous experimental work.

While the grain mixtures used for the different cows in the same trial varied only slightly as to the per cent of total nitrogen, the per cent of total nitrogen as non-protein nitrogen varied considerably, from 6.41 per cent to 15.05 per cent in trial 1, from 9.10 to 18.01 per cent in trial 2, from 7.89 per cent to 11.44 per cent in trial 3, and from 7.30 to 19.08 per cent in trial 4. These variations could not be correlated with the make-up of the various grain mixtures, nor did any reasonable explanation for them present itself. Many of the analyses were repeated several times without changing the values first obtained.

The results given in table 2 and the data referred to in the dis-

[4] Albuminoid method.

[5] Foreman alcohol titration method.

cussion of these results must also raise questions as to the general usefulness of the published data on the non-protein nitrogen content of feeds. If the method itself is accurate and thus roughages may vary as greatly as the data show, tables giving the "true protein" content based on average analyses for non-protein must have a large probable error. That large variations may exist according to stage of maturity and other factors is reasonable to expect, as has been discussed. However, the data obtained for the grain mixtures raise a question as to the accuracy of the method itself—a question which limits the value of the following discussion and which should be borne in mind, although the extent of its effect cannot be evaluated.

In terms of the rations fed, it was found that the timothy hay ration in every case, except for cow 2, trial 2, contained more non-protein nitrogen than did the clover hay ration (column 5, figures for "ration"). The per cent of total nitrogen as non-protein nitrogen was greater in every case on the timothy hay ration than on the clover hay ration (column 6, figures for "ration"). The per cent of total nitrogen of the ration as non-protein nitrogen contributed by the hay and silage was quite uniform in each trial (column 8), and was greater throughout on the timothy hay ration. While both types of rations furnished approximately the same amount of total protein, the timothy hay ration furnished the greater proportion of non-protein.

In column 9 data are given showing the non-protein nitrogen in the feces of each cow. Since these figures are in all cases very much smaller than those for non-protein nitrogen in the ration, as shown in column 5, it may be concluded that some at least of this non-protein nitrogen was digested. Digestion coefficients may be computed showing a digestibility of 60 to 80 per cent in most cases. Such figures cannot be considered accurate, however, both because there is no way of knowing to what extent this non-protein nitrogen in the feces originated from protein in the feed, and because some of the non-protein nitrogen in the feed may have been built into protein by bacteria yet not have been digested. It seems reasonable, nevertheless, to conclude that a large percentage of the non-protein nitrogen in the feed was di-

gested, at least as well as the crude protein which was found to be from 55 to 65 per cent digestible by Maynard, Miller and Krauss (1).

Was this digested non-protein nitrogen utilized for the performance of any protein function? This cannot be proved, but there is presumptive evidence that it was:

The results reported by Maynard, Miller and Krauss (1) show a positive nitrogen balance in nine out of twelve cases where the digestible crude protein was generally insufficient to supply the maintenance requirement according to the Morrison standard plus the nitrogen in the milk. In view of this efficient utilization of digestible crude protein it seems reasonable to assume that the 15 to 20 per cent of it that was non-protein in nature was used to some extent at least.

Table 2 shows more non-protein nitrogen in the timothy ration than in the clover ration, yet according to Maynard, Miller and Krauss the nitrogen of the timothy ration was at least as well utilized as that of the clover ration.

Certain additional evidence is to be found in the discussion that follows of the partition of the nitrogen in the urine.

In table 3 data are presented showing the partition of nitrogen in the urine. Table 4 contains the same data expressed in per cent of the total nitrogen. The outstanding feature of these data is the large amount of undetermined nitrogen, showing that a relatively large amount of nitrogen is present in some form other than ammonia, urea, and hippuric acid. The same situation was found to exist in the work of Lindsay (26) in which, when only the urea and hippuric acid nitrogen are considered, 25.4 per cent of the total nitrogen remains as undertermined. In order to determine, if possible, the principal constituent contributing to the large amount of undetermined nitrogen, a sample of urine from a cow in another trial not reported in this paper, was analyzed for creatinine and creatine. Only a trace of creatinine was found, but 25 per cent of the total nitrogen was present as creatine nitrogen. Based upon Lindsay's work, in which 5.0 per cent of the total nitrogen in the urine was reported as creatinine, we would expect some creatinine to be present also. The reason

TABLE 3

Partition of nitrogen in the urine

TRIAL	COW	VOLUME EXCRETED	N PER 100 CC.	TOTAL N	AMMONIA N	UREA N	HIPPURIC ACID N	UNDETERMINED N
		cc.	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
1	1	7,518	0.8301	62.41	*	31.43	11.85	19.13
	2	10,460	0.6732	70.42	*	31.07	11.61	27.74
	3	7,523	1.1880	89.37	*	44.69	12.71	31.97
2	1	5,540	1.2524	69.38	*	27.87	14.39	27.12
	2	7,216	1.0406	75.09	*	38.10	12.15	24.84
	3	6,359	1.5226	96.82	*	48.46	14.37	33.99
3	4	5,508	1.0894	60.00	4.82	22.91	11.93	20.34
	5	5,079	1.0053	51.06	3.84	15.09	14.06	18.07
	6	5,860	0.9877	57.88	2.99	25.14	12.59	17.16
4	4	8,000	0.6575	52.60	9.20	8.08	11.18	24.14
	5	8,214	0.6138	50.42	2.83	20.62	10.90	16.07
	6	8,674	0.6474	56.16	2.43	21.95	10.45	21.33

* Not determined.

TABLE 4

Percentage partition of nitrogen in the urine

TRIAL	COW	TOTAL N	AMMONIA N	UREA N	HIPPURIC ACID N	UNDETERMINED N
		grams	*per cent*	*per cent*	*per cent*	*per cent*
1	1	62.41	*	50.35	18.99	30.66
	2	70.42	*	44.12	16.49	39.39
	3	89.37	*	50.00	14.23	35.77
2	1	69.38	*	40.16	20.74	39 09
	2	75.09	*	50.74	16.17	33.09
	3	96.82	*	50.05	14.84	35.11
3	4	60.00	8.03	38.19	19.88	33.90
	5	51.06	7.51	29.54	27.54	35.41
	6	57.88	5.16	43.43	21.76	29.65
4	4	52.60	17.49	15.36	21.26	45.89
	5	50.42	5.62	40.89	21.63	31.86
	6	56.16	4.33	39.08	18.61	37.98

* Not determined.

practically none was found may be that since creatinine is gradually changed to creatine when in contact with alkalies, and since cow urine is alkaline, the creatinine may have been hydrolyzed to creatine. This would also explain the large amount of creatine found. Presumably allantoin nitrogen was also present as a part of the undetermined nitrogen since Lindsay found 6.3 per cent of the total nitrogen in this form.

The tables indicate that while a greater volume of urine was excreted on the clover hay rations than on the timothy hay rations, there was no significant difference in the amount of total nitrogen and urea nitrogen excreted. Less total and urea nitrogen were excreted in trials 3 and 4 because the protein intake was less. The small amount of urea found in the urine of cow 4, trial 4, was probably due to the fact that this sample was improperly preserved, resulting in the breaking down of some of the urea into ammonia as indicated by the relatively large amount of ammonia found.

A small amount of ammonia was found. This is contrary to the results of Lindsay who found practically no ammonia. However, it is in accord with Salkowski (27) who found 0.38 gram of ammonia per liter in fresh cow urine. The differences in the amount of ammonia found in trials 3 and 4 are not great enough to be of significance.

It will be seen from table 3 that without exception slightly more hippuric acid nitrogen was excreted on the timothy rations than on the clover rations. While this suggests that a greater amount of benzoic acid and benzoic acid-like substances were present in the timothy rations, the difference is not great enough to be conclusive, as other factors, particularly bacterial decomposition of proteins, affect the amount of benzoic acid eliminated.

A study of the urine analyses indicates no difference in the protein metabolism on the two types of rations.

SUMMARY

Data are presented showing the distribution of non-protein nitrogen in dairy rations based upon clover and timothy hay, the digestibility of the non-protein nitrogen, and its utilization by

cows in milk. The results of a study of the partition of the nitrogen in the urine collected while these rations were being fed are included. The following conclusions are based on these data:

1. The non-protein nitrogen in hay and silage varies greatly in different samples, presumably due to differences in stage of maturity, method of curing, and other factors. This large variation suggests that figures for true protein based on average analyses are of limited usefulness.

2. The albuminoid nitrogen method of the Association of Official Agricultural Chemists is of doubtful accuracy for distinguishing between crude and true protein in feedingstuffs.

3. Dairy rations made up of hay, silage, and grain, contain a relatively large amount of non-protein nitrogen. This nitrogen is apparently useful in meeting a part of the protein requirement of a cow in milk.

4. There is no difference in the protein metabolism when timothy hay or clover hay is fed, as indicated by urine analysis.

The writer desires to express his appreciation for the generous assistance and valuable suggestions given by Dr. L. A. Maynard, both in conducting the experimental work and in preparing this paper. He also wishes to express his appreciation to Dr. J. B. Sumner for his valuable suggestions as to methods for urine analyses and for the use of his laboratory, and to Dr. R. C. Miller for his generous assistance in conducting the experiment.

REFERENCES

(1) Maynard, L. A., Miller, R. C., and Krauss, W. E.: Cornell Univ. Memoir, 1927.
(2) Armsby, H. P.: U. S. D. A. Bur. of An. Ind. Bul. 139, 1911.
(3) Armsby, H. P.: The Nutrition of Farm Animals, 1917, pp. 684–688.
(4) Henry, W. A., and Morrison, F. B.: Feeds and Feeding, 1923, p. 64.
(5) Forbes, E. B.: Jour. Dairy Sci., 1924, vii, 154–159.
(6) Mitchell, H. H.: Natl. Res. Council Bul. 55, 1926, p. 7.
(7) Völtz, W.: Biochem. Zeitschr., 1920, cii, 151–227.
(8) Salkowski, E.: Zeitschr. f. Physiol. Chem., 1920, cix, 276–279.
(9) Morgen, A., Schöler, G., Windheuser, K., and Ohlmer, E.: Landw. Vers. Sta., 1921, xcix, 1–26.
(10) Morgen, A., Windheuser, C., and Ohlmer, E.: Landw. Vers. Sta., 1922, xcix, 359–366.

(11) Völtz, W., Dietrich, W., and Jantzon, H.: Biochem. Zeitschr., 1922, cxxx, 323–431.
(12) Honcamp, F., Koudela, S., and Müller, E.: Biochem. Zeitschr., 1923, cxliii, 11–155.
(13) Morgen, A., Windheuser, C., and Ohlmer, E.: Landw. Vers. Sta., 1924, ciii, 1–40.
(14) Honcamp, F., and Schneller, E.: Biochem. Zeitschr., 1923, cxxxviii, 461–496.
(15) Schwarz, C.: Biochem. Zeitschr., 1925, clvi, 130–137.
(16) Folin, O., and Youngburg, G. E.: Jour. Biol. Chem., 1919, xxxviii, 111–112.
(17) Folin, O., and Bell, R. D.: Jour. Biol. Chem., 1917, xxix, 329–335.
(18) Hawk, P. B.: Practical Physiological Chemistry, 7th ed., p. 519.
(19) Van Slyke, D. D., and Cullen, G. E.: Jour. Biol. Chem., 1914, xix, 211–228.
(20) Kingsbury, F. B., and Swanson, W. W.: Jour. Biol. Chem., 1921, xlviii, 13–20.
(21) Folin, O.: Jour. Biol. Chem., 1914, xvii, 469–473.
(22) Grindley, H. S., and Eckstein, H. C.: Jour. Amer. Chem. Soc., 1916, xxxviii, 1425–1431.
(23) Annett, H. E., and Russell, E. J.: Jour. Agr. Sci., 1908, ii, 382–391.
(24) Shaw, R. H., Wright, P. A., and Deysher, E. F.: U. S. D. A. Bul. 953, 1921.
(25) Woodman, H. E., and Amos, A.: Jour. Agr. Sci., 1924, xiv, 99–113.
(26) Lindsay, D. E.: Biochem. Jour., 1912, vi, 79–95.
(27) Salkowski, E.: Zeitschr. f. Physiol. Chem., 1904, xlii, 213–250.

THE CREAM PLUG*

ITS CAUSES AND PREVENTION

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One of the problems met in the selling of bottled cream is the formation of the cream plug. This cap or plug which frequently gathers on the cream varies in tenacity from the consistency of heavy cream to a leathery condition of such firmness that it prevents the spilling of the cream when the bottle is inverted. The undesirability of such cream plugs is self-evident. A waste of butter fat occurs when they are discarded as they usually are; but from the milk dealers' standpoint the chief objection is the unfavorable impression such bottled cream makes on the consuming public.

Two attempts to gather information on this problem have been made in the form of questionnaires sent to milk dealers. These surveys of opinions served to emphasize the importance of the problem, but contributed little to its solution, for they failed to reveal the fundamental factors involved in it. In the experimental work here reported, the main aim was to determine these fundamental factors.

THE COMPOSITION AND MICROSCOPIC APPEARANCE OF CREAM PLUGS

In order to determine the exact nature of the cream plug, analyses and microscopic examinations were made. The plugs from two sources were analyzed; some were taken from returned University cream and others from the cream of a local company. These plugs were analyzed for fat by ether extraction and tested for total solids, by drying to constant weight at 100°C.

* The experimental work was done by Mr. K. M. Royer under the fellowship established by the International Association of Milk Dealers. Published with the permission of the Director of the Wisconsin Agricultural Experiment Station. Received for publication May 1, 1927.

The analyses of the cream plugs show that they consist principally of fat. From our knowledge of the normal rising of fat in milk, we would hardly expect such a rich accumulation of

TABLE 1

The composition of cream plugs

SAMPLE NUMBER	PER CENT FAT	PER CENT SOLIDS-NOT-FAT	PER CENT WATER
1	77.60		
2	79.80		
3	62.05	4.49	33.46
4	65.07	2.10	32.83
5	55.40	5.30	39.30
6	58.10	4.10	37.80
7	61.40	4.00	34.60
Average.....	65.57		
Average excluding 1 and 2.....	60.40	4.00	35.60

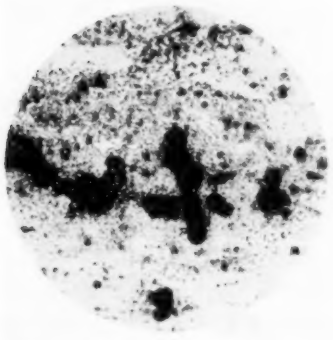


FIG. 1. LOW POWER PHOTOMICROGRAPH OF CREAM PLUG, 70 PER CENT FAT

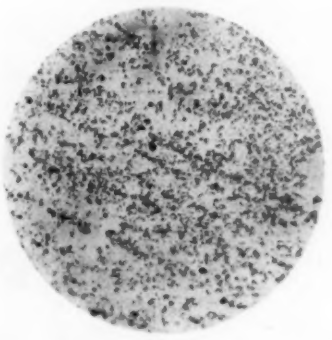


FIG. 2. LOW POWER PHOTOMICROGRAPH OF CREAM, 70 PER CENT FAT

fat if the fat were still in normal emulsion. Microscopic examination of the cream plugs readily revealed that the fat was no longer in normal emulsion but had coalesced into irregular masses of fat. (Compare figures 1 and 2.)

THE FUNDAMENTAL CAUSE OF CREAM PLUG FORMATION

From the general knowledge that emulsions are less stable as the size of the globules increases and that the large globules rise more rapidly than small globules, the logical deduction is that the formation of cream plugs is dependent upon the size of the fat globules. The large fat globules rise more rapidly and are more likely to coalesce than the small fat globules. A more exact understanding of the relative velocities with which globules of different sizes rise, can be obtained by an application of Stokes equation. Ruling out those factors in this equation which are constant or over which we have no control, we find that the velocity with which the globules rise is directly proportional to the square of their radius, and inversely proportional to the viscosity of the liquid through which they are rising. Thus the size of the fat globules and the viscosity of the serum of the cream may be expected to be factors in cream plug formation.

Observations made in the course of the experimental work are in harmony with these deductions.

FACTORS THAT AFFECT THE SIZE OF FAT GLOBULES

Since the size of the fat globules determines whether the cream will develop cream plugs, it becomes of importance to consider the factors that affect the size of the fat globules. The effect of breed and stage of lactation on the size of the fat globules in the milk are well known. Similarly, the possibility of partial churning of milk while being transported especially in partly filled cans and the tendency of freezing to collect the fat, need only be mentioned here. No further experimental proof is required on these factors.

The experimental work conducted concerned itself mainly with demonstrating the effect of various types of agitation to which the milk and cream may be subjected in the milk plant.

METHOD OF MEASURING THE SIZE OF CREAM PLUGS

To measure the tendency of the various experimental cream samples to form plugs, the weight of the plug was obtained.

This was considered satisfactory since the toughness of the plug was in general proportional to the weight.

A number of special cups were made of perforated sheet metal as shown in figure 3. One of these cups was placed into each bottle of cream immediately after bottling. After the bottled cream had been stored as desired the plug was then removed by slowly raising this cup out of the bottle by means of the handle which projected slightly above the surface of the cream. The cup was allowed to drain a standard length of time, and the weight of the material retained in it was then determined.

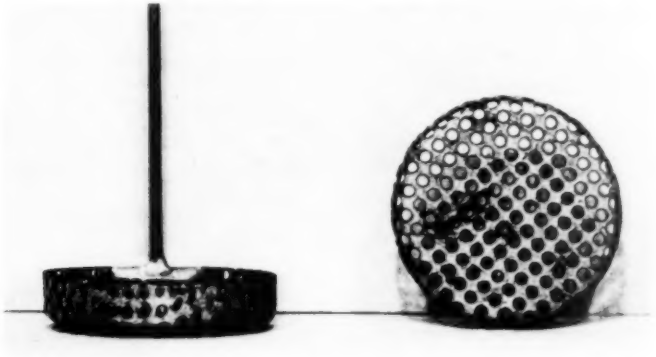


FIG. 3. CUP USED IN REMOVING CREAM PLUGS

THE EFFECT OF AGITATION

In order to demonstrate the effect of agitation on cream plug formation, 25 per cent cream was agitated at 45°F. by pouring from one pail into another 25 times. Samples of this agitated cream and from another portion of the same cream that had not been so agitated were set aside as described above, and after forty-eight hours the weights of the cream plugs were determined (table 2). Samples of the fresh agitated and unagitated cream were also taken for microscopic examination. Photomicrographs were taken of these samples in a 1:50 dilution in neutral 3 per cent gelatin (fig. 4).

The results obtained demonstrate that the agitation caused the fat to collect into larger globules and increased cream plug formation.

TABLE 2

The effect of agitation on cream plug formation

WEIGHTS OF CREAM PLUGS FROM:	
Agitated cream	Unagitated cream
grams	*grams*
5.5	5.5
7.1	3.9
6.9	4.3
6.0	4.8
6.6	3.2
Average.....6.42	3.94

TABLE 3

The effect of bottling foamy cream on cream plug formation

Cream agitated, poured 25 times

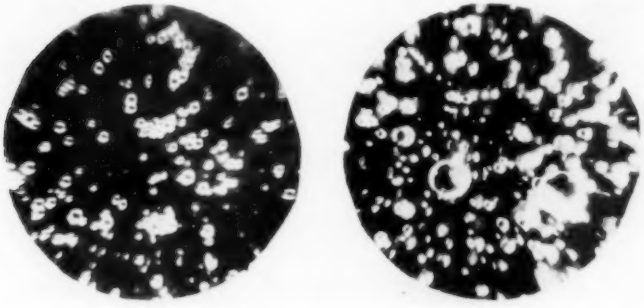
WEIGHTS OF CREAM PLUGS FROM:	
Foamy cream	Air removed by vacuum
grams	*grams*
14.3	6.6
13.8	6.5
13.4	7.2
13.0	7.1
15.7	4.9
11.8	5.5
11.6	7.6
14.5	8.1
12.5	7.5
11.7	7.4
Average.....13.2	6.8

In another similar experiment the agitated samples (37 samples) averaged 2.26 grams heavier cream plugs than the unagitated samples (39 samples).

In these two experiments where cream was agitated cold by

pouring it was necessary to eliminate any air that was incorporated, by subjecting the cream to a vacuum for five minutes, as preliminary trials had shown that if any foam was not eliminated a foamy accumulation would interfere with the measurement of the true cream plug.

Since under commercial conditions a similar condition might at some time be encountered, the results obtained when the foam was not eliminated, are given in table 3. The cream used was unpasteurized 25 per cent cream, agitated by pouring from one



a *b*

FIG. 4. *a*, PHOTOMICROGRAPH OF NORMAL CREAM; *b*, PHOTOMICROGRAPH OF AGITATED CREAM

pail to another 25 times. The foamy plugs were not so close in texture and not so firm as the usual plugs, but nevertheless they were objectionable.

THE EFFECT OF AGITATION AT DIFFERENT TEMPERATURES

In order to determine at what temperatures the effect of agitation is most pronounced, a series of experiments was conducted similar to the above, using cream heated to temperatures of 50°, 100°, 120° and 140°F. Air was eliminated as in the other experiments. The results given in table 4 show that agitation at the lower temperatures is most harmful.

That agitation at pasteurizing temperature has little harmful

effect is further demonstrated by the results given in table 5 where the weight of the plugs is given for samples of cream taken from a coil vat pasteurizer at three different intervals,—imme-

TABLE 4

The effect of agitation at different temperatures on cream plug formation

EXPERIMENT NUMBER	WEIGHTS OF CREAM PLUGS FROM CREAM				
	Agitated at 140°F.	Agitated at 120°F.	Agitated at 100°F.	Agitated at 50°F.	Not agitated
I	3.8	3.1	4.1	5.6	3.0
	3.1	3.4	4.7	5.0	3.2
	2.6	2.9	3.4	6.5	2.4
	2.8	4.5	3.4	4.6	2.9
II	3.9	3.1	4.6	5.9	3.3
	3.3	4.5	3.4	5.2	3.5
	3.9	4.0	4.0	5.2	1.9
	3.6	3.6	4.3		
Average.....	3.4	3.7	4.0	5.5	2.9

TABLE 5

The effect of agitation at pasteurizing temperature on plug formation

Stored three days at cooler temperature

WEIGHTS OF CREAM PLUGS:		
Cream heated to 145°F.	After 20 minutes agitation at 145°F.	After 30 minutes agitation at 145°F.
2.6	3.3	3.1
2.9	5.0	4.0
3.0	5.0	4.9
3.5	4.6	4.3
3.0	4.0	3.8
Average.....3.0	4.4	4.0

diately after the cream had been heated to 145°F., after having been held at 145°F. for twenty minutes, and after thirty minutes.

The effect of agitation at lower temperatures is further demonstrated in an experiment in which a lot of cream was divided into

two lots; one lot was pasteurized and cooled in a coil vat pasteurizer; the other lot was heated to 145°F. by means of a flash pasteurizer, held at 145°F. for thirty minutes and then cooled over an aerator. The former of these two lots of cream was thus subjected to considerable agitation in the heating and cooling, while in the latter lot this agitation was reduced to a minimum. The results given in table 6 show that reducing the agitation to a

TABLE 6

The effect of reducing agitation to a minimum while heating and cooling

WEIGHTS OF CREAM PLUGS FROM CREAM:	
Pasteurized and cooled in coil vat	Flashed to 145°F., held 30 minutes, cooled over aerator
9.0	3.0
9.1	2.8
9.6	4.5
6.7	3.5
7.0	4.1
9.3	4.2
8.6	3.8
9.3	4.5
8.6	4.3
8.3	4.1
8.5	4.0
9.7	5.1
9.8	3.6
8.7	5.0
9.5	4.4
Average.....8.78	4.06

minimum while heating and cooling the cream materially reduces cream plug formation as compared with heating and cooling as in a coil vat pasteurizer.

A consideration of the results given in tables 4, 5, and 6 leads to the conclusion that agitation of cream at any temperature tends to increase cream plug formation, and that agitation of cream at lower temperatures favorable to churning is decidedly more harmful than agitation at pasteurizing temperatures.

THE EFFECT OF AN UNBALANCED SEPARATOR BOWL IN SEPARATING THE CREAM

The separator bowl being out of balance is frequently mentioned as a cause of cream plug formation. In order to determine whether the vibration and possible agitation to which cream would be subjected in an unbalanced separator bowl would have any effect on cream plug formation, an experiment was conducted in which a lot of milk was divided into two lots,—one lot was

TABLE 7

The effect of an unbalanced separator bowl on cream plug formation

EXPERIMENT NUMBER	WEIGHT OF PLUG	
	Unbalanced bowl	Balanced bowl
	Skim milk test 0.13 per cent	Skim milk test 0.075 per cent
I	3.0	2.6
	2.0	2.1
	2.4	1.2
	2.0	2.0
	1.7	1.7
	1.7	2.2
	Skim milk test 0.118 per cent	Skim milk test 0.042 per cent
II	4.0	3.0
	2.6	3.4
	3.5	4.0
	3.5	3.2
	3.7	3.7
	4.6	4.6
Average	2.9	2.8

separated by means of a cream separator with the bowl running true, and the other lot was separated by the same separator after the bowl had been unbalanced by placing a small sheet of lead between two of the plates in the separator bowl. The two lots of cream were separately standardized, pasteurized and bottled. The results of two such experiments are given in table 7. The fat test of the skim milk in each case is given as an indication of the fact that the bowl was unbalanced. The results demon-

strate that the presence of a cream plug on bottled cream cannot be attributed to an unbalanced separator bowl.

THE EFFECT OF STORAGE TEMPERATURE ON CREAM PLUG FORMATION

In considering the fundamental causes of cream plug formation it was pointed out that two important factors that determine the

TABLE 8

Temperature of storage and cream plug formation

Held four days, 20 per cent cream

WEIGHT OF PLUG	
Room temperature 70°F.	Cooler temperature 42°F.
grams	*grams*
15.10	6.10
14.00	7.37
17.95	5.37
17.69	6.17
15.85	4.62
11.17	7.53
18.07	6.36
8.75	5.30
18.67	5.20
17.10	4.68
15.40	5.43
17.92	3.35
17.87	5.75
19.57	5.27
15.75	6.81
20.94	9.48
Average.....15.94	5.92

rise of fat globules are the size of the globules and the viscosity of the serum. In the foregoing the effect of the size of the fat globules has indirectly been considered as affected by agitation of the cream.

The viscosity of the serum as a factor in this problem is controllable only in so far as temperature affects the viscosity. It is commonly known that the viscosity of fluids increases as the

temperature is lowered. It is to be expected therefore that storing the cream at low temperatures would reduce the rise of the globules and reduce cream plug formation.

In an experiment to demonstrate the effect of storage temperatures, pasteurized 20 per cent cream was bottled and bottles stored at 42° and 70°F. for four days. The weights of the plugs formed were then determined. While there had been a noticeable increase in the acidity of the cream at 70°F. the cream had not thickened, so that the results given in table 8 represent the weight of the actual plugs (see fig. 5). At 70°F. plugs were formed that were very tough and leathery and were approximately three

a *b*

FIG. 5. *a*, SHOWS AVERAGE CREAM PLUG FORMED AT COOLER TEMPERATURE; *b*, SHOWS AVERAGE CREAM PLUG FORMED AT ROOM TEMPERATURE

times as heavy as those formed at 42°F. That this difference in the weight of plugs at the two temperatures is to be attributed to the physical effect of the temperature difference and not to the bacterial growth and acidity differences was demonstrated in experiments in which it was found that there was no difference in cream plug formation at room temperature between samples of different acidities and between preserved and unpreserved samples.

EVAPORATION AND CREAM PLUG FORMATION

It has been suggested that cream plug formation may be due to evaporation from the surface of the cream. The analyses of

the cream plugs lend some strength to this suggestion, although the results given in table 1 are not extensive enough to warrant drawing any positive conclusion on this point. Furthermore, even if these results were accepted as conclusive, the high solids-not-fat content might be due largely to adsorption.

In order to determine to what extent evaporation might be a factor in cream plug formation a comparison was made in which thirty bottles of cream from the same lot were divided into three

TABLE 9

The effect of evaporation from capped bottles on cream plug formation

	WEIGHT OF PLUGS		
	Paraffined cap	Usual cap	Porous paper cap
	grams	*grams*	*grams*
Room temperature storage; time, two days	15.5	14.5	16.2
	13.1	14.8	15.8
	15.4	14.0	15.7
	13.0	15.6	15.5
	12.8	13.3	16.2
Average	13.96	14.44	15.88
Cooler temperature storage; time, two days	6.6	6.4	5.6
	6.6	5.7	7.0
	5.5	6.7	6.8
	6.1	4.4	6.5
	5.4	3.9	7.0
Average	6.04	5.42	6.58
Grand average	10.00	9.93	11.23

lots and capped as follows: Lot 1 was capped with the usual cap and further sealed by carefully paraffining over the cap; lot 2 was capped with the usual cap only; and lot 3 was covered with two thicknesses of porous paper toweling held in place with rubber bands. Each of these three lots was subdivided into two lots of five bottles each and stored at room temperature and at the cooler temperature (40° to 45°F.). After two days the cream plugs were determined in the usual manner. The results given in table 9 show that evaporation was only a slight factor. In-

cidentally these results again emphasize the importance of storage temperature in preventing cream plug formation.

REMEDIES FOR CREAM PLUG FORMATION

The above results and discussion indicate that the prevention of cream plug formation resolves itself into handling the milk and cream in such a manner as to prevent the fat from coalescing into large globules and clusters of fat. In practical operations this means reducing agitation of the milk and cream to a minimum, especially at temperatures that are favorable to churning. While it is impossible to enumerate all objectionable practices a few of the more common ones are as follows:

1. Transporting milk in partly filled cans. The extent of churning taking place will depend upon the amount of shaking to which the cans are subjected in transit and the temperature of the milk. Milk improperly cooled, at temperatures of 60°, 70° and even 80°F. will churn more than milk properly cooled at 50°F., or lower.

2. Freezing of the milk. This is known to cause the partial coalescence of the fat.

3. Delayed heating of the cream in a coil vat pasteurizer. In some instances the cream may be run into a coil vat, agitated with the coil and not promptly heated to pasteurizing temperature. This is injurious since any delay means that the cream will be subjected to more agitation than necessary and this is at a low temperature, which is most harmful.

4. Running the coil too rapidly.

5. Having the coil vat pasteurizer only partly full. The agitation in such cases is more severe than in a full pasteurizer.

6. Cooling the cream in the vat pasteurizer with the coil. In all cases cooling by running the cream over an aerator or through an internal tubular cooler is preferable because of the decidedly smaller amount of agitation involved.

7. Repasteurizing of the cream.

In addition to the practices mentioned above and which concern themselves with the coalescing of the fat into larger masses, the storage temperature of the bottled cream must be mentioned as

important. The higher the storage temperature, the less viscous the serum of the cream will be, and the more rapid and the more pronounced the cream plug will be.

If the milk and cream is obtained from such sources that the milk dealer has little or no control over the past history of these products, or where the exercising of the proper control from the standpoint of cream plug formation is difficult or impossible, remedies other than preventative must be employed. In such cases cream plugs can still be eliminated by applying the knowledge that the fundamental factor is the presence of large fat globules, by reducing the size of the fat globules by means of the centrifugal emulsifier or by means of the homogenizer.

By experiment it was found that the emulsifier and the homogenizer would eliminate the undesirable cream plugs. In using the homogenizer it is to be remembered that the higher pressures will reduce the whipping quality of the cream and increase the tendency of the cream to "feather" when added to coffee or other hot liquid. Pressures should be used that are just sufficient to accomplish the desired result.

The exact pressure necessary depends upon the condition of the cream, the make and mechanical efficiency of the homogenizer. Pressures ranging from 200 to 1000 pounds per square inch are being used for this purpose.

SUMMARY

The cream plugs contain a high percentage of fat and microscopic examination shows that the fat has coalesced into irregular masses. The fundamental cause of cream plug formation is the presence in the cream of large fat globules which will rise rapidly into a dense layer and coalesce. This rising and coalescing of these fat globules is most rapid at higher storage temperatures because of the fact that the serum through which the globules must rise is less viscous.

The prevention of cream plug formation, therefore, resolves itself into handling the milk and cream in such a manner that partial churning is avoided, or if this is impossible, reducing the size of the fat globules by either emulsifying or homogenizing at

low pressures. Storing the cream at proper storage temperatures is helpful in preventing the formation of the cream plugs, but this is beyond the control of the milk dealer after it leaves his plant and, therefore, should not be relied upon to any great extent.

MAINTENANCE REQUIREMENTS FOR CALVES TESTED BY LIVE WEIGHT METHODS*

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In connection with our studies of growing cattle the question of the requirements of the animal for maintenance has often arisen. Armsby (1) is the only authority who has published tables of estimated maintenance requirements including ranges of weights normal to calves under one year of age. The figures for the smaller weights were calculated by using his extensive results from mature animals. The question of the accuracy of these figures first arose as the result of an experiment in which a heifer made a gain of approximately a pound per day for six months while receiving a ration, the net energy value of which, when calculated by the Armsby method, was approximately the amount set forth as the maintenance requirement in his tables.

The limited value of data professing to deal with maintenance requirements taken by the live weight method is fully recognized especially when the subjects are immature animals. However, it is believed the data to be presented have some value as an indication of the extent to which maintenance figures calculated from mature animals are applicable to young animals.

PROCEDURE

Three procedures might be used in a study of this problem. The first is to feed an animal a given percentage of the energy estimated as the maintenance requirement, using Armsby's maintenance figures and method of calculating energy values.

The second is to vary the feed from day to day as appears necessary to maintain a uniform weight for a period of time with a determination later of the energy used during the trial.

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The third is to feed a constant ration which has been computed from a preliminary period to supply sufficient energy for maintenance. If the estimate is too low the animal will lose weight until the ration is sufficient for maintenance. If too high, the animal will gain in weight until an equilibrium is reached. Experience indicated the third plan to be most satisfactory and it was used exclusively during the latter part of the study.

The first plan required that the ration be adjusted at frequent intervals to allow for any change in the weight of the animal. Variations from day to day in weight due presumably to fill often result when this plan is followed in changes being made in the ration that later are found to have been a mistake. The same difficulty of knowing the true weight of the animal interferes with the details of operating the second plan. The third plan of feeding a constant ration throughout the maintenance period eliminates some of these difficulties and if the ration chosen is close to the true maintenance requirement the animal will soon adjust itself to it by a gain or loss in weight and thereafter remain quite constant.

It is recognized that with mature animals the length of the trial is an important factor regarding the dependability of the results. With growing animals it is not advisable to undertake to keep the animal at a constant weight for any length of time. Waters (2) found that profound physiological changes occur in young animals that are fed a limited ration. With animals of this age the live weight may readily remain the same while the animal is not in energy equilibrium. Experience indicated that about forty days was the most satisfactory period for use with the young calf while for older animals longer periods are feasible.

ANIMALS AND RATIONS USED

Fifteen animals were used, all of the Holstein breed, of which five were males and ten females. The males were used in the lighter weights only and on the plan where a constant ration was used. Alfalfa hay and wheat straw were used for roughage. In order to keep a growing animal quiet on a limited ration,

experience indicated it was necessary to feed a bulky ration which will insure a normal amount of material in the alimentary tract. To secure the desired bulk with alfalfa alone would in some cases provide more energy than was desired. By using wheat straw as a low net energy filler it was possible to make such adjustments as necessary in energy intake and still keep the animals reasonably contented. The grain ration used was uniformly a mixture of ground corn 4 parts, wheat bran 1 part, linseed oilmeal 1 part.

In making the advance calculations of the ration to be fed average values were used in most cases as the chemical analyses were generally not available until later. All feeds used except the skimmilk were analyzed by the Division of Agricultural Biochemistry. From these data the net energy values were calculated according to Armsby's method. The digestion coefficients as given by Morrison (3) were applied to the chemical analyses. Factors given by Armsby (1, p. 650) were used for the calculation of the therms metabolizable energy. The factors for the increment of heat production were those recommended by the same author (1, p. 652). A protein supply was provided at all times considerably above the amount usually specified as the minimum required for maintenance.

The animals were kept in individual stalls bedded with shavings and allowed the freedom of a dry lot a part of the day, when the weather was not too severe. Water was offered once daily in the barn and was available in the exercise lot. Salt and bone meal were available in each stall. Each animal was weighed daily before the morning feed.

EXPERIMENTAL

Experiment 1. Three animals approximately six months old at the beginning were used in this trial. An attempt was made by frequent adjustments to provide 100 per cent of the Armsby maintenance requirement. However, as the calculations for adjusting the ration were made by using average analyses of the feeds included and the analyses later available showed somewhat more than average value the ration actually received

was a trifle higher than intended. The results are shown in figure 1. The animal E-12 received 101.6 per cent for the 130 days, E-14, 101.4 per cent and E-17, 101.9 per cent of Armsby's standard for the 115 days on experiment. Two of the animals gained about half a pound daily and the third somewhat more.

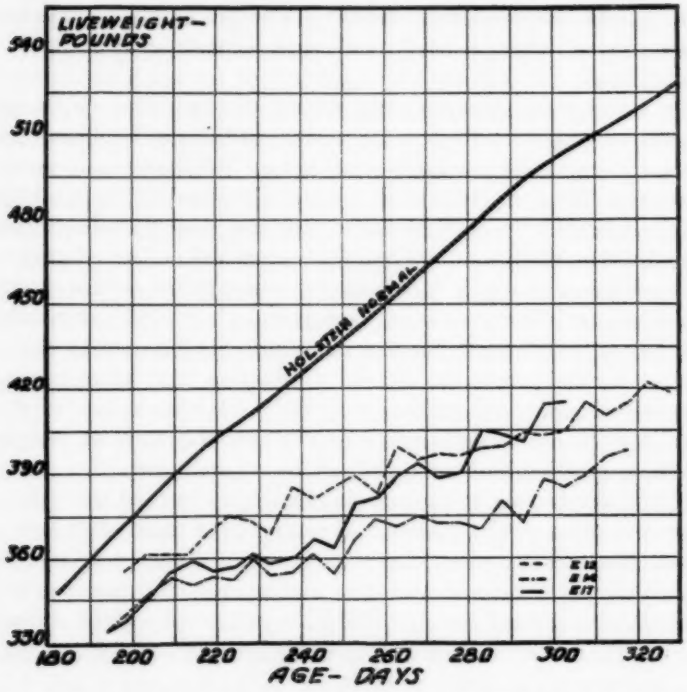

FIG. 1. RESULTS FROM SUPPLYING CALVES WITH 100 PER CENT NET ENERGY NEEDED FOR MAINTENANCE ACCORDING TO ARMSBY'S TABLES

The three heifers receiving a net energy intake, as prescribed by Armsby, gained over half a pound daily for the period of the trial, 110 to 120 days.

These results confirmed our previous experience and indicated the energy for maintenance of small animals as specified by Armsby's tables is unnecessarily high when calculated by the present net energy values.

Experiment 2. Four animals were used ranging in age from 100 to 271 days at the beginning of the experiment. An attempt was made to keep the weights of the animals constant by adjusting the rations at frequent intervals although the results were only partially successful. The fact that in every case, although the ration given was below 100 per cent of Armsby's standard, all the animals maintained their weight or made gains indicated the Armsby standard calls for more energy than these animals required.

Experiment 3. In this experiment the third plan of procedure already described was followed—that of estimating on the basis of a preliminary period what feed intake would be necessary for maintenance then continuing to feed this ration without change throughout the period. If the ration as estimated proved to be more liberal than maintenance a gain would presumably be made until equilibrium was established. If too small the same adjustment would occur at a point somewhat below the original weight.

Data were taken from 21 periods using 11 different animals. In those cases where the animal was used twice the first period was followed by one of liberal feeding and the second trial was not undertaken until the animal was again approximately normal in weight for its age. In all cases a preliminary period was used for adjusting the ration to a point which appeared to result in a stabilization of weight. However in some cases the amount of feed necessary to maintain the animal was misjudged during the preliminary period and as the plan followed was to make no changes in ration during the trial period gains or losses in certain cases were too much to justify the use of the data obtained. In table 1 is presented a summary of the results from 18 periods taken with 11 individuals in trials where the weight remained essentially the same. The first 16 represent animals in normal condition of flesh and the last two animals in very high condition. These data show that all calves in condition described as normal were maintained on a ration averaging 90 per cent of the Armsby specification. In only one of the 16 trials was 100 per cent of Armsby's maintenance figures reached and this

is apparently accounted for by a condition of the animal above normal.

Condition as a factor. The last two figures in the table give the results of a maintenance period of two calves in high flesh

TABLE 1

Summary of all trials resulting in weight maintenance

NUMBER OF ANIMAL	LIVE WEIGHT		LENGTH OF TRIAL	NET ENERGY RECEIVED PER DAY	NET ENERGY PER 1000 POUNDS	PLANE OF NUTRITION	CONDITION OF ANIMAL
	Initial	Gain for period					
	pounds	*pounds*	*days*	*therms*	*therms*	*per cent*	
E-79	87	0	20	1.16	5.91	98.5	Normal
E-87	117	−3	25	1.33	5.56	92.7	Normal
E-79	135	0	25	1.41	5.36	89.3	Normal
391	233	−1	40	1.75	4.62	77.0	Normal
390	249	−2	45	1.94	4.90	81.7	Normal
391	275	4	35	2.29	5.41	90.2	Normal
390	308	1	15	2.48	5.44	90.7	Normal
E-15	323	2	35	2.55	5.42	90.3	Normal
E-16	394	0	40	2.94	5.47	91.2	Normal
E-65	416	−1	35	3.11	5.58	93.0	Slightly high
E-55	455	0	40	3.20	5.41	90.2	Normal
E-54	468	2	40	3.21	5.32	88.7	Normal
E-65	537	0	25	3.15	4.77	79.5	Thin
E-53	663	1	25	4.26	5.60	93.3	Slightly high
E-46	798	1	40	5.18	6.02	100.3	Above normal
E-53	861	2	35	5.48	6.06	101.0	Above normal
Average.....		.375	32.5		5.43	90.5	
E-55	257	1	25	2.84	7.03	117.1	Very fat
E-54	284	−2	40	2.83	6.55	109.1	Very fat
Average.....			32.5		6.79	113.1	

First group of 16: mean average therms net energy required per 1000 pounds, 5.43 ± 0.066; standard deviation 0.390 ± 0.046.

Including all, mean average therms net energy required per 1000 pounds. 5.58 ± 0.090; standard deviation 0.569 ± 0.064.

at the time. It will be noted in both cases the requirements were decidedly above Armsby's figures.

These two animals had been on a high plane of feeding, including whole milk, previous to the experimental period and were

as a result in a high condition of flesh. This condition is further shown by the fact that E-55 was 22.4 per cent and E-54, 33.6 per cent above normal weight at the beginning of the trial.

One of these heifers as shown by the figures in table 1 required 109 and the other 117 per cent of Armsby's estimated energy requirement to maintain weight. The evidence that condition is the factor responsible is further strengthened by the results of using the same animals later. The figures in the same table show that at an age of about nine months the same animals were maintained with a ration supplying 89 per cent of Armsby's requirement which is in line with the results from the others.

GENERAL DISCUSSION

It is possible the ability of the dairy calf to maintain itself on a ration somewhat below Armsby's calculated amounts is to be attributed to the use in his experiments of animals of the beef type, normally in higher condition than dairy type animals. The evidence presented in this report indicating the importance of condition corroborates much of the evidence on the subject as summarized by Armsby and Moulton (4). Furthermore the results reported in the present paper are in line with the recent results of Cochrane, Fries and Braman (5) regarding the energy requirement for maintaining three Jersey cows. Two mature cows were maintained on approximately 90 per cent of the Armsby figure while one especially quiet animal used only about 70 per cent.

The revision of the net energy value now under way as announced by Forbes will affect the calculations of these trials. Alfalfa furnished a portion of the nutrients in nearly all the rations and assigning a higher value to alfalfa will raise the plane of nutrition beyond that calculated in this study. A recalculation will be desirable after the revision is completed.

One factor of economic importance indicated by this study is the increased energy required for maintaining growing heifers in high condition. On the other hand care should be taken not to go to the other extreme lest the animals be underfed to the extent of affecting the size adversely.

CONCLUSIONS

The net energy (calculated by the Armsby method from the chemical analyses of feed used) necessary to maintain uniform weight in dairy bred calves in normal flesh is about 90 per cent of that set forth in Armsby's Tables.

REFERENCES

(1) ARMSBY, H. P.: The Nutrition of Farm Animals, p. 711. Macmillan Co., New York, 1917.

(2) WATERS, H. J.: The capacity of the animal to grow under adverse conditions. Proceeding Soc. Promotion of Agricultural Science, 29, 71–96. 1908.

(3) MORRISON, F. B., AND HENRY, W. A.: Feeds and Feeding, Ed. 18, p. 728. Madison, Wisconsin, 1923.

(4) ARMSBY, H. P., AND MOULTON, C. R.: The Animal as a Converter of Matter and Energy, p. 181. Chemical Catalog Co., New York, 1925.

(5) COCHRANE, D. C., FRIES, J. A., AND BRAMAN, W. W.: The Maintenance Requirement of Dry Cows. Jour. Agr. Research, xxxi, 1055–1083, 1925.

A STUDY OF "FLAKY" MILK*

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It has become the practice in certified dairies to examine the milk from each cow before each milking. The usual procedure consists in drawing three streams from each teat upon a brass screen containing 100 meshes to the inch. If the milk passes readily through the screen and no residue remains, the secretion is regarded as normal; if however particles or floccules remain on the screen, the milk is rejected. Thus gross abnormalities in the secretion are readily detected, since the presence of flocculi is regarded as one of the principal signs of udder derangement. In a considerable number of instances floccules in small numbers are observed in the milk but no palpable abnormalities are found in the mammary gland. The general appearance of the secretion from such cases is not greatly altered except for the presence of relatively few, irregular, elongated, tiny, white particles. The floccules may be present for a single milking only or they may occur during a few days, disappear, and recur after varying intervals. That the condition is of considerable economic importance is borne out by the fact that a large number of cases occurred in a large herd during 1926, necessitating a considerable loss of milk to the producer. The condition is of considerable interest from the standpoint of the public health, since slight abnormalities in the character of the milk afford the most practicable and most rapid method for the detection of udder disease. Hence a knowledge of the true nature of the particles and their significance is essential. With these points in view the milk and the udders of a number of cases were examined.

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METHODS

At the start we examined the milk from the involved quarters of 20 consecutive cases as they occurred in a large herd. They were those reported to the herdsman by the foremilker. The samples for examination were obtained by one of us before the next regular milking. The udder was cleansed and the milk was drawn directly from the udder and immediately brought to the laboratory for examination. A mixed sample from the uninvolved quarters was usually examined. The procedure employed was to dilute 1 cc. of the milk with 9 cc. sterile NaCl solution. 0.5 cc. of the mixture was then plated with 0.5 cc. defibrinated horse blood and 10 cc. agar. Ten cubic centimeters of the milk was centrifuged rapidly for 10 minutes and blood agar slants in series inoculated wih a large loop of the sediment. Films were also prepared from the sediment. The plates were examined after twenty-four and forty-eight hours incubation. The fresh milk was always poured through a soil sieve of 100 meshes to the inch and any residue washed gently with NaCl solution. Particles were thus rendered easily visible and could be picked from the screen and examined microscopically. In addition quantities of milk varying from 25 to 50 cc. from 13 cases were centrifuged at high speed and the sediment, after suspending in sterile broth, injected into the peritoneal cavities of young guinea pigs. A sample was saved and when possible coagulated with rennet and the resulting whey, or the whole milk, tested for B. abortus agglutinin. The brief history given with the protocols of the cases was obtained from the herd records after the case had been studied. Inasmuch as the general features of the condition are similar only a few cases will be mentioned in detail.

STUDY OF CASES

Case 1425. The milk from the left hind quarter was reported to contain flakes on January 5, 1927. Abnormalities of the quarter were not noted. The milk contained large numbers of tiny, elongated, white particles composed of leucocytes and mucus, and others made up of epithelial cells. The centrifuged sample revealed considerable yellow,

tenacious sediment composed largely of leucocytes in masses. The milk contained 2200 bacteria per cubic centimeter; 95 per cent of them were hemolytic staphylococci. The agar slant cultures from the sediment developed hemolytic staphylococci. The milk from the other three quarters appeared normal and failed to contain the staphylococci.

Case 1426. On July 13, 1926, mastitis developed in the right hind quarter. On August 1 and 2 and December 21, 26, and 27, the milk from the left front quarter was flocculent. Mastitis developed in this quarter on February 16, 1927. On December 22, 1926, the quarter appeared slightly firmer along the ventral aspect. The milk contained small floccules composed of sheets of epithelial cells. On December 27, there were a considerable number of elongated flocculi composed of leucocytes and mucus. There was considerable sediment made up of leucocytes. Micrococci were found in the films both free and within phagocytes. The plate cultures revealed 9540 hemolytic staphylococci per cubic centimeter. The same organism had been found on December 22. It was also present in the attack of mastitis on February 16, 1927.

Case 1427. Abnormalities in the milk from the left fore quarter were noted March 9, November 30, and December 11, 1926. On February 4, 1927, clinical mastitis developed in this quarter. Examination of the quarter on December 12, 1926, failed to reveal abnormalities. The milk on that day contained small flakes of epithelium. It was more watery in appearance than that from the other quarters. There was considerable yellow sediment composed of large numbers of leucocytes and cells of the endothelial type. The plate revealed 1000 non-hemolytic streptococci per cubic centimeter in the quarter. The milk from the other three quarters did not contain the streptococcus nor was an abnormal amount of sediment noted in the centrifuged sample.

Case 1428. On January 9, 1927, the milker reported flocculi in the milk from the left fore quarter. The sample on January 10 failed to show flakes. Plate cultures revealed 3840 B. lactis aerogenes per cubic centimeter. The centrifuged sample revealed an excessive amount of sediment composed of masses of leucocytes. On January 11, clinical mastitis developed, the milk was purulent, and the bacilli were isolated from the plates.

ANALYSIS OF THE FINDINGS IN 20 CASES

In our series of 20 cases the bacteriological findings were as follows:

From 9, non-hemolytic streptococci
From 5, hemolytic streptococci
From 4, hemolytic staphylococci
From 1, B. lactis aerogenes
From 1, nothing significant

As a rule the organisms were in moderate numbers, rarely above 9000 per cubic centimeter. In most instances udder pathogens appeared in blood agar plate cultures containing 0.05 cc. of milk. However, in some cases relatively few organisms grew in the plates, but the blood agar slant cultures prepared from the sediment revealed a type capable of giving rise to udder disturbance. In one instance neither the plate cultures nor the horse blood agar slants revealed a suggestive organism. However, sufficient time had elapsed between the attack and our examination to warrant the presumption that any pathogen may have been completely eliminated from the udder.

In 7 cases it was possible by palpation to detect abnormalities in the quarter. They consisted of irregular, firm areas, either diffuse or sharply circumscribed, in the region of the cistern or about the larger ducts. In 13 we failed to find such abnormalities. Five cows of the series developed mastitis subsequent to our examination.

It is of further interest to note that 7 cows had no previous history of disturbance in the quarter involved prior to our examination; 4 had two attacks, 1 three, 3 four attacks, 3 others had 5 attacks, 1 had been reported six times, and another on ten occasions.

The floccules observed on the sieve vary considerably in appearance and number. Those apt to arouse the attention of the fore milker are usually elongated, verminous-like particles, tenacious in character. In general they are 1 or 2 mm. in length, although at times some may reach a length of 5 mm. When these are washed free from casein, they are found to be composed of densely

packed masses of leucocytes, apparently held together by mucus. As a rule they are covered with fat globules, but when the particle is crushed between coverslip and slide and examined microscopically its true nature is apparent. Their tenacious character and castlike appearance are characteristic. Particles of this nature were readily recognized in our samples, doubtless this type is characteristic of the condition. In a single instance distinctly visible floccules of mucus were obtained from the sieve. The sieve residue from many samples of milk revealed minute, irregular, flattened particles. These on examination proved to be small sheets of flattened, squamous, epithelial cells. They are evidently exfoliated particles from the lining of the lower teat canal. Examination of scrapings from the teat canal below the sphincter revealed cells of similar character and arrangement. They may be disregarded as having pathological significance since they evidently result from mechanical pressure during milking. Evidently they are too small to be seen during the routine examination of fore milk, or they strike the sieve with such force as to be broken and pass through.

It is obvious that the condition we have described is an udder inflammation of a mild character. The organisms encountered are capable of producing a severe mastitis and are similar to those noted previously by one of us (1). It is of further significance that 5 of the cows developed clinical mastitis at varying periods after our examination. In these instances the organism present during the mild attack, characterized by the appearance of a few flakes, was likewise overwhelmingly predominant in the severe clinical condition. However, it may be said that although a sufficiently pathogenic organism was present, its numbers were too small to be of much significance. A single case may be cited to show that considerable variation in the number of organisms occurs from time to time even though the milk is drawn at the same hour and by the same individual each day.

Case 1429. January 10, 1927. Left fore quarter. Milk contained characteristic floccules. There were 540 non-hemolytic streptococci per cubic centimeter. There was considerable sediment composed of

leucocytes. The milk from the other three quarters was normal and contained 260 organisms per cubic centimeter.

January 11. Flocculi were present in the milk from the left fore quarter. There was an excessive amount of sediment composed of leucocytes. The streptococci numbered 7640 per cubic centimeter. The milk from the other three quarters was normal, and although examined daily never showed abnormalities nor contained streptococci throughout our observations.

January 12. Floccules of leucocytes were present in the milk from the left fore quarter. Leucocytes were found in large numbers in the sediment. A total count of 260 bacteria per cubic centimeter was obtained; 30 per cent were streptococci.

January 13. No flakes were observed in the milk from the left fore quarter; there was, however, the usual excessive amount of sediment. The count rose to 5120 non-hemolytic streptococci per cubic centimeter.

January 14. No floccules were present, but 5900 streptococci per cubic centimeter were found.

January 15. Floccules were found and the count fell to 600 streptococci per cubic centimeter.

January 16. No floccules; 5640 streptococci per cubic centimeter.

January 17. Floccules; 2040 non-hemolytic streptococci per cubic centimeter.

January 18. No floccules; 480 non-hemolytic streptococci per cubic centimeter.

Other cases similar in character have been studied with much the same result. There apparently exists no correlation between the actual number of streptococci and the presence of the floccules. The protocol further brings out the point previously mentioned that the number of organisms may be so scarce at certain times that they may be overlooked.

It might also be suggested that there existed within the udder an organism capable of inciting a mild inflammation which could not be cultivated by the methods outlined. To test this possibility, we inoculated 13 guinea pigs, averaging 250 grams in weight, with all the sediment obtained from centrifuging 25 to 50 cc. of milk drawn directly from the involved quarter. The animals were under close observation for ten days following inoculation

and as far as we could tell remained well. Thirty five to forty days later they were killed and agar slants inoculated with bits of spleen. In no instance was B. abortus or other suggestive organism isolated. In addition the milk from all involved quarters failed to agglutinate B. abortus in sufficiently high titer to suggest severe udder infection with this organism. As a further confirmation, films from all sediments were fixed and stained. As a rule the bacteria encountered on microscopic examination could be cultivated. In a number of instances phagocytosis of streptococci and staphylococci was encountered and affords presumptive evidence that such organisms were producing sufficient irritation to arouse the defensive mechanism.

DISCUSSION

The microscopic and bacteriological findings are suggestive that the irregular appearance of elongated, tenacious particles in the milk is associated with bacterial inflammation. The organisms encountered are similar to those usually met with in bovine mastitis. In a number of instances palpaple lesions, usually consisting of irregular, ill defined, firm areas in the ventral portion of the quarter or in the region of the cistern and large ducts, suggested a chronic inflammation. This is well borne out in many instances by the number of attacks extending through one or more lactation periods. However, in the majority of instances lesions are not apparent. The excessive number of leucocytes and the increase in the titer of the blood serum proteins in the milk are indicative that inflammation exists. The castlike character of the floccules argues that certain small lactiferous tubules are involved. The irregular appearance of the floccules in the milk is suggestive that under certain conditions leucocytes are bound together by mucus and may represent casts of some of the small ducts; due to mechanical or other causes they are released and finally reach the cistern. The readiness with which they are detected depends entirely on their size. The tenacious character of the milk sediment in cases where no visible flocculi occur, suggests that the aggregates

of leucocytes and mucus are too small to be detected with the unaided eye.

In addition to these points, samples of relatively fat-free milk from 14 affected quarters were tested with cow serum precipitin by the method described by one of us (2). In every instance the milk reacted well above the level of normal milk, showing that sufficient inflammation existed to produce the increased elimination of blood serum proteins.

From the history and our findings in many instances, it is apparent that these cows are relatively resistant. The organisms as a rule are held pretty well in check; if this were not the case the whole udder would soon become involved. In a number of cases this resistance was in some way overcome and in consequence the quarter became clinically involved. Occasionally a quarter may become infected, flakes appear in the milk during a few milkings, and the organism disappear from the quarter. This would explain the case in which no pathogen was found.

Of our series of cases it may be said that we were dealing with several well defined degrees of resistance. Those animals which have one attack and recover must be regarded as most resistant; those which have several attacks and show no definite lesions are perhaps less resistant, the third group which have had several attacks and whose udders show certain abnormalities are on the whole still less resistant; and finally the group whose resistance was overcome by the infecting agent afford a further example of at least a temporary condition in which the resistance was largely overcome. It must be recognized that such cases as we have described are as dangerous from the standpoint of spreading udder infection as severe clinical cases.

It may be said that the organisms which we have encountered are not regarded as pathogenic for humans. However, it is possible that organisms from human sources gaining access to the udder may set up a mild inflammation not essentially different from the type encountered. With this in view we regard the usual method of withholding the milk of such cows from the general supply as essential.

SUMMARY

A condition characterized by the irregular appearance of tiny, elongated, tenacious floccules in the milk of certain cows is described. In the majority of instances the involved quarter presents little that is abnormal; in certain cases firm areas about the cistern and milk ducts were noted. The floccules characteristic of the condition are composed of masses of leucocytes and mucus. Organisms of the same types (non-hemolytic and hemolytic streptococci and hemolytic staphylococci) as those found in mastitis are present in the "flaky" milk. An increased number of leucocytes and a relatively high blood protein elimination are characteristic. The findings suggest a mild but prolonged mastitis.

REFERENCES

(1) JONES, F. S.: Jour. Exper. Med., 1918, xxviii, 149.
(2) JONES, F. S.: Jour. Exper. Med., 1926, xliii, 451.

CAMEMBERT CHEESE FROM PASTEURIZED MILK*

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INTRODUCTION

The quality and uniformity of Camembert cheese is determined to a great extent by the quality of the milk used from day to day. Pasteurizing the milk has been shown to improve the quality of Cheddar cheese. Since faulty flavors and textures in Cheddar and Camembert cheese are probably caused by similar microörganisms, it seems very possible that pasteurization of the milk can also be successfully applied in the manufacture of Camembert cheese.

Previous attempts to make cheese from pasteurized milk

Numerous investigators have attempted to apply pasteurization as a means of reducing or eliminating the daily changes in the process of cheesemaking, which are largely due to variation in the biological properties of the milk.

Dunne (1) in 1918 reported that in 1908 and 1909 a large number of dairies in Denmark produced hard cheese from pasteurized milk for the chief purpose of ridding the milk of pathogenic bacteria. O. Wennevold was the promoter of the idea of pasteurizing the milk for cheesemaking in Denmark. In 1915 he stated that pasteurizing temperatures of 149° to 158°F. were becoming general and gave good results.

Atkinson (2) in 1922 found that a better cheese was obtained by the "holder" than by the "flash" method. Practically no difference was observed in the time of coagulation when the milk was pasteurized at 145°F. for thirty minutes and a slightly larger amount of starter added. The flavor of the cheese was superior and the yield greater.

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Murray (3) in 1923 obtained similar results.

Gow (4) in 1922 reported that in South America Gouda cheese was manufactured successfully from milk pasteurized by the holder method.

Stevenson (5) in 1923 stated that the manufacture of cheese from milk heated to a temperature of 160° to 165°F. for an instant, cooled, and set with rennet, without the addition of acids or salts, was common in New Zealand. The advantages in favor of pasteurized milk cheese were: Improved flavor, greater uniformity, larger yield, better keeping quality, and simplified process of manufacture.

Price (6) in 1927 partially reviews investigations previous to the year 1922. In his own experiments he showed that milk pasteurized at 145°F. for 30 minutes produced a better quality of cheese than any other holder methods of pasteurization tried.

Kneuttinger (7) in 1926 found that pasteurization of the milk produced inferior Tilsit cheese. He stated that a bitter flavor resulted, perhaps from the influence of the heat upon the albuminoids and salts of the milk, or the increased retention of whey in the curd.

In 1926 Marquardt and Hucker (8) reported that cheese made from pasteurized milk by the holder method showed the greatest improvement when the milk was produced under satisfactory sanitary conditions.

Previous attempts to make Camembert cheese from pasteurized milk

Little has been done to find out whether pasteurized milk can be made successfully into Camembert cheese.

Dunne (1) previously mentioned, reported in 1918 that during the war Denmark produced good Camembert cheese from pasteurized milk to supply Scandinavia.

Golding (9) in 1913 made cheese from pasteurized milk in attempts to improve the keeping quality and to prevent gassy fermentation. Pasteurization temperatures of 150°F. were used. The quality was found to be decidedly improved and the yield slightly greater.

Selection of pasteurizing method

It has been shown that pathogenic and other undesirable organisms commonly found in milk are destroyed or inactivated by exposure to a temperature of 145°F. for thirty minutes. Practical experience has also proved that satisfactory Cheddar cheese can be produced from milk pasteurized by the holder method. This method does not injure the coagulability of the milk, since a good coagulation following this treatment can be obtained without the addition of chemicals.

These facts led to the use of the holder method of pasteurizing in the following experiments.

PURPOSE AND PROCEDURE OF EXPERIMENTS

Purpose of experiments

There were 25 lots of cheese made. Lots 1 to 15 were intended to determine the correct conditions for curdmaking. Based upon their results lots 16 to 25 were planned to determine the effect of the pasteurization upon the quality of the cheese.

Plan of procedure

The milk which was used in the 25 paired experiments originated from different sources and consisted of various grades. In each of the lots 1 to 15, 80 pounds of milk was equally divided and placed in two vats. Each vat of milk was made into 10 cheese. In lots 16 to 25 the amount of milk used was increased to 120 pounds and 15 cheese made out of each vat of milk. Four pounds of milk were required for each individual cheese which weighed approximately ½ pound. In each experiment one of the two vats of milk was pasteurized at 145°F. for thirty minutes and cooled to setting temperature. Enough extra milk was added to each vat to make two rennet tests before and after the addition of the commercial lactic acid starter. Previous to renneting the milk was inoculated with a pure culture of Penicillium camemberti, grown on sterilized crackers. Each vat of milk was made into the best cheese possible. The cheese were cured in the manner recommended by Thom and Fisk, Matheson, and Sammis (10, 11, 12).

Testing

The samples of milk, whey, green and ripened cheese were analyzed for fat and total solids by the Mojonnier method and apparatus.

Scoring of cheese

The scoring of the cheese was done by seven members of the staff of the Department of Dairy Industry. The identity of the cheese was unknown to them. Since there was no standard Camembert cheese score, the following score card was selected as a means of determining the quality of the cheese. Perfect score for: Flavor 50, body and texture 30, mold growth 10, color 5, finish 5, total 100.

To determine the quality of each lot of cheese three raw and three pasteurized milk cheese were selected and presented to the judges. The average of the scores for the three raw and the three pasteurized milk cheese from each lot was taken to indicate the actual quality of the cheese.

Interpretation of results

"Student's" (13) method of interpreting the data of the paired experiments was used. By this method the odds that a given difference is significant are stated as 30 to 1 or 500 to 1, and so on. Odds of 30 to 1 in this work are held to indicate a significant difference.

Student's (13) tables were modified by Love (14). Love's tables were used in this work.

EXPERIMENTS

Effect of pasteurizing the milk on the score of the cheese

These data were obtained from 23 paired experiments. The differences between the average scores of the two types of cheese are summarized in table 1, which shows also the probable error and the coefficient of variation of the scores. From this table it seems evident that a higher scoring cheese can be produced

from pasteurized milk than from identical raw milk. The coefficient of variation tends to show that the quality of the pasteurized milk cheese is more uniform.

In lots 1 to 15 the difference between the scores of the paired observations is 4.82 points, and in lots 16 to 25 3.88 points. The odds that the differences are significant are 197:1 and 100:1 respectively.

The pasteurized milk cheese was inferior in quality to the raw milk cheese in only three out of the 23 experiments. In these instances accidental contamination of the pasteurized milk cheese was responsible for their inferior quality.

TABLE 1

Quality of the cheese made from raw and pasteurized milk

LOT NUMBER	NUMBER OF OBSERVATIONS	TREATMENT	MEAN SCORE	COEFFICIENT OF VARIATION
1–15	13	Raw*	81.48 ± 1.62	10.2
		Pasteurized*	86.30 ± 0.89	5.3
16–25	10	Raw*	78.13 ± 1.11	6.3
		Pasteurized*	82.01 ± 0.67	3.6

* All cheese made from the same milk received the same lot number with the differentiation of "raw" and "pasteurized."

Influence of the quality of milk on the score of the pasteurized milk cheese

In comparing the scores of the raw and pasteurized milk cheese, it was noticed that the difference seemed to be greatest when the raw milk cheese was of inferior quality.

The coefficient of correlation between the raw milk cheese scores and the gain in score of the pasteurized milk cheese in lots 1 to 25 was found to be -0.782 ± 0.054. This indicates that with increasing score of the raw milk cheese, the difference in score between it and the pasteurized milk cheese becomes smaller.

Further it was noticed that with decreasing score of the raw milk cheese the score of the pasteurized milk cheese also de-

creases. To verify this observation the coefficient of correlation was determined and found to be $+0.732 \pm 0.065$.

If it is assumed that the quality of the raw milk cheese indicates in a general way the quality of the milk from which it was made, it seems logical to conclude from these observations that pasteurization of the milk is most effective when the raw milk is of inferior quality.

Effect of starter on the quality of the curd

The firmness and the acid development of the curd are the factors which determine to a large extent the proper draining and ripening of Camembert cheese. In making cheese from raw milk it is desirable to increase the acidity of the milk by adding starter to such an extent that the proper acid development and firmness of the curd for cutting is reached in one an one-quarter to one an three-quarters hours after setting the milk.

The appearance of whey drops on the surface of the curd is the generally accepted sign that the curd is ready to be dipped into the hoops. In these experiments it was found that this condition was obtained in the average quality raw milk with the addition of 3 to 4 per cent of clean-flavored, sixteen-hour-old starter. Due to the fact that in the milk pasteurized at a temperature of 145°F. for thirty minutes, most of the lactic acid producing organisms are destroyed, it is evident that a larger amount of starter is required. In the course of the experiments it was noticed that 4 to 5 per cent of starter brought about the desired conditions of the curd made from pasteurized milk.

Influence of rennet on the quality of the curd

Thom and Fisk, Sammis, and Matheson (10, 11, 12) recommend 3 to 5 ounces of rennet per 1000 pounds of milk. In these experiments it was found that 4 ounces of rennet gave a good coagulation of the milk and the proper firmness of the curd. To determine whether a greater amount of rennet would be beneficial in making Camembert cheese from pasteurized milk, three experiments were made with increased amounts of rennet. In

all 3 cases the effect of the greater amount of rennet on the quality of the curd was unsatisfactory. The curd had the tendency to be too firm, and the expulsion of the whey was slow. Very little or no sweating of the curd at the time of dipping, with slow draining of the cheese in the hoops, was noticed.

Effect of pasteurization of the milk upon the yield of Camembert cheese

All the cheese made were weighed every day, from the first day after making until they were wrapped in tinfoil and boxed. The final weight was taken immediately before scoring and disposing of the cheese. Scales graduated to 0.02 pound were used. The yield of green cheese was calculated on the basis of 100

TABLE 2

Average amount of cheese from 100 pounds of milk and starter

LOT NUMBER	TREATMENT	GREEN CHEESE	RIPENED CHEESE
		pounds	*pounds*
1–13	Raw	21.45	14.02
	Pasteurized	22.25	14.17
16–25	Raw	19.38	13.47
	Pasteurized	19.25	13.41

pounds of milk and starter after draining from twenty to twenty-four hours. The yield of ripened cheese was determined in the same manner at the time of scoring.

Table 2 is a summary of the observations.

The experiments show that the average yield for raw and pasteurized milk cheese is practically the same.

Effect of pasteurization on the shrinkage of the cheese

The amount of weight lost by the cheese during the process of curing is influenced largely by the loss of moisture, which depends chiefly on the moisture content of the cheese, the temperature and humidity of the curing room, the length of time of curing of the cheese, and the rapidity and intensity of the mold growth previous to wrapping and boxing.

These factors were constant for each pair of cheese, but some variations occurred in some respects in the curing of different pairs. A record was kept of the loss of weight for lots 1 to 25, and the percent of shrinkage was calculated.

The loss of weight of the pasteurized milk cheese is greater in lots 1 to 15. This is explained by the fact that in lots 1 to 8 the same amount of starter was used in each pair of the raw and pasteurized milk cheese. At the time of dipping and draining the pasteurized milk cheese in each lot had a tendency to retain more moisture than the raw milk cheese, due to the slower development of acid.

In lots 16 to 25, where the amount of starter added to the pasteurized milk is properly adjusted, the loss of weight is practically the same in both types of cheese.

TABLE 3

Loss of weight of raw and pasteurized milk cheese

LOT NUMBER	TREATMENT	PER CENT OF WEIGHT OF GREEN CHEESE LOST IN CURING
1–15	Raw	34.52
	Pasteurized	36.31
16–25	Raw	29.86
	Pasteurized	30.05

Effect of pasteurization on the fat, total solids, and solids not fat content of the cheese

The green cheese made from pasteurized milk contains more moisture in lots 1 to 15. This accounts for the lower per cent of fat in the cheese. In lots 16 to 25 the composition of both types of cheese is practically the same. The data tend to show that with the proper method of curdmaking it is possible to obtain the same composition in the pasteurized milk cheese that is desired in the raw milk cheese. The percentage of fat, total solids, and solids not fat increases during the ripening process due to the loss of moisture.

To determine whether more fat, total solids, and solids not fat

are retained in the cheese made from pasteurized milk, the pounds of fat, total solids, and solids not fat are calculated for the cheese obtained from 100 pounds of milk and starter and summarized in table 5.

The amounts of fat, total solids, and solids not fat retained in the cheese made from pasteurized milk are slightly greater only

TABLE 4

Fat, total solids, and solids not fat tests of green and ripened cheese made from raw and pasteurized milk

LOT NUMBER	TREATMENT	FAT		TOTAL SOLIDS		SOLIDS NOT FAT	
		Green	Ripened	Green	Ripened	Green	Ripened
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1–15	Raw	18.48	25.59	34.49	46.09	16.01	20.50
	Pasteurized	18.34	25.63	34.10	46.50	15.76	20.87
16–25	Raw	19.12	26.40	36.32	49.06	17.20	22.66
	Pasteurized	19.19	26.84	36.49	49.63	17.30	22.79

TABLE 5

Distribution of fat, total solids, and solids not fat in raw and pasteurized milk cheese, made from 100 pounds of milk and starter

LOT NUMBER	TREATMENT	FAT		TOTAL SOLIDS		SOLIDS NOT FAT	
		Green	Ripened	Green	Ripened	Green	Ripened
		pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1–15	Raw	3.95	3.60	7.36	6.47	3.44	3.34
	Pasteurized	4.04	3.62	7.49	6.58	3.48	3.33
16–25	Raw	3.69	3.56	7.00	6.60	2.87	2.98
	Pasteurized	3.69	3.58	7.00	6.59	2.92	2.97

in lots 1 to 15. The differences are not large enough, however, to be significant.

Effect of pasteurization upon the keeping quality of the cheese

After scoring, some representative samples of each lot of cheese were kept at a temperature of 53°F. for one to four weeks,

to determine whether there existed any difference in the keeping quality of the cheese.

The results did not lead to any definite conclusion. The keeping quality of the cheese is determined largely by the quality of the milk, the moisture content of the cheese, and the mold growth. Where these three factors were nearly the same, practically no difference in the keeping quality of the cheese could be observed. However, it was noticed in the course of the experiments that faulty flavors apparent at the time of scoring were more pronounced when the cheese were held for two to four weeks. Bitter flavor especially seemed to increase with the age of the cheese.

TABLE 6

Fat, total solids, and solids not fat lost in the whey

LOT NUMBER	TREATMENT	FAT	TOTAL SOLIDS	SOLIDS NOT FAT
		per cent	*per cent*	*per cent*
1–15	Raw	0.169	0.659	0.490
	Pasteurized	0.116	0.654	0.537
16–25	Raw	0.089	0.657	0.568
	Pasteurized	0.071	0.668	0.597

Effect of pasteurization upon the fat, total solids, and solids not fat content of the whey

Most of the whey drippings were collected at the time of dipping the curd. After the cheese had drained for one and one-half to 2 hours, representative samples of the whey collected from the raw and pasteurized milk were analyzed.

The results in lots 1 to 25 show that the average per cent fat lost in the whey from raw milk is greater than in the whey from pasteurized milk. The mean difference in lots 1 to 15 is 0.053 per cent, and in lots 16 to 25 0.018 per cent. The odds that the differences are significant are 1666:1 and 1999:1 respectively. The total solids content in the whey from both types of cheese in lots 1 to 25 is so variable, that the differences +0.005 for lots 1 to 15 and −0.011 for lots 16 to 25 are insignificant, since the odds are only 5.27:1 and 5.52:1 respectively. The calcu-

lated loss of solids not fat is greater in the whey from pasteurized milk. The mean differences are 0.047 for the lots 1 to 15 and 0.028 for lots 16 to 25. The odds that these differences are significant are 2247:1 and 1666:1 respectively.

CONCLUSIONS

1. Camembert cheese can be successfully made from milk pasteurized at a temperature of 145°F. for thirty minutes.
2. Pasteurized milk produces a cheese of better average quality and uniformity.
3. The improvement of the quality of cheese is greatest when the raw milk previous to pasteurizing is of inferior quality. The quality of the pasteurized milk cheese improves with improving quality of the raw milk.
4. In making Camembert cheese from pasteurized milk a larger amount of starter should be used.
5. Pasteurization has practically no influence upon the yield of ripened Camembert cheese.

REFERENCES

(1) Dunne, J. J.: Pasteurized milk for cheesemaking. Hoard's Dairyman, vol. 55, p. 466, 1918.
(2) Atkinson, T. H.: The pasteurization of milk for cheese manufacture. New South Wales, Dept. Agr., Hawkesburry Agr. College, 1922. Printed by Alfred J. Kent, Govt. Printer, Sydney.
(3) Murray, J. K.: Pasteurization of milk for cheddar cheese making. Agr. Gaz. N. S. W., vol. 34, 1923, no. 8, pp. 559–566.
(4) Gow, J. P.: Gouda cheese manufactured from pasteurized milk. N. Y. Prod. Rev. and American Cry., vol. 53, no. 10, pp. 470–472, 1922.
(5) Stevenson, C.: Pasteurization of milk for cheddar cheese making in New Zealand. Proc. of World's Dairy Congress, 1923, pp. 306–308.
(6) Price, W. V.: The manufacture of cheddar cheese from milk pasteurized by the holder method. Memoir 105, April, 1927, Cornell University, Ithaca, N. Y.
(7) Kneuttinger: (Kiel 1925) Pasteurization of milk for cheesemaking. Molkerei Ztg., vol. 40, no. 14. Abstract in Creamery and Milk Plant Monthly, May, 1926, pp. 54–55.
(8) Marquardt, J. C., and Hucker, G. J.: The effect of pasteurization and cooling of milk upon the quality of cheddar cheese. N. Y. State Agr. Exp. Sta. Bul. 534, Geneva, March, 1926.

(9) GOLDING, N. S.: Better Camembert cheese from pasteurized milk. Ontario Agr. College, Annual Report 29, pp. 68–70, 1913.
(10) THOM, CHARLES, AND FISK, W. W.: The book of cheese, pp. 117–130, 1918.
(11) MATHESON, K. J.: The manufacture of Camembert cheese. Dept. of Agr. Dairy Division, Bureau of Animal Industry, Bul. 1171.
(12) SAMMIS, J. L.: Cheesemaking, pp. 151–152, 1924.
(13) STUDENT: Biometrika, vol. 6 (1908), pp. 1–25; vol. 11 (1917), pp. 414–417.
(14) Love, H. H.: A modification of Student's tables. Jour. Amer. Soc. Agronomy, vol. 16, no. 1, January 1924.

BACTERIOLOGICAL METHODS OF EXAMINING ICE CREAM*

FOREWORD

The American Dairy Science Association, through its committee on bacteriological methods, is contemplating the formulation of a complete set of bacteriological procedures useful in controlling the quality of all dairy products. This committee expects to act largely through sub-committees appointed from men in the organization who have had experience with the bacteriological analysis of various dairy products. Obviously, the formulation of such methods should not be left to the arbitrary decision of a committee, but should be the result of suggestions and criticisms coming from all interested parties, whether or not they are members of the American Dairy Science Association. The committee, therefore, wishes to serve as a center about which methods satisfactory to the largest number may be evolved.

The purpose of this preliminary report is to submit to the ice cream industry at large, proposed methods of sanitary control of ice cream. These will be revised later in accordance with the suggestions received and the judgment of the committee, before they are finally adopted by the American Dairy Science Association and included in the report on bacteriological methods of analyzing dairy products.

AGAR PLATE METHOD OF ICE CREAM ANALYSIS

Preparation and sterilization of equipment

Glassware. All glassware should be sterilized in the hot air sterilizer at 170° to 180°C. for from one to two hours. Pipettes and Petri dishes should be wrapped in paper[1] or enclosed in metal containers to prevent contamination upon removal from the

* Received for publication May 20, 1927.

[1] Imported Kraft wrapping paper withstands sterilizing temperatures excellently without charring.

sterilizer. In arranging the material in the hot air sterilizer, care should be taken to permit free circulation of air about each package or piece of glassware.

Method of sampling

Liquids. Cream, milk, skim milk or ice cream mix should be sampled by means of a sterile sampling tube. Such a tube can be made by flaming the ends of a 36-inch piece of ½-inch glass tubing. Aluminum or other metal tubes may also be used. One to four tubes may be completely wrapped in paper or the tubes may be enclosed in a metal case before sterilization. If the cream or milk to be sampled is in cans, a composite sample may be obtained by agitating, and then slowly inserting the tube to the bottom of each can, removing, thereby, a sector of the contents and placing in a sterile sample bottle. The sample bottle best adapted to this work is a wide mouth, ground glass stoppered bottle of about 120-cc. capacity. Fifty to 100 cc. of sample should be taken in each instance.

If the liquid to be sampled is in a vat, the contents should be agitated thoroughly, then sampled by means of the sterile sampling tube in at least six places (preferably more) in the vat.

Liquid samples taken from the spout of the homogenizer or freezer should be obtained by quickly passing the bottle under the opening at periodic intervals during the discharge so as to get representative samples of the material delivered from the machine.

Solids. Sugar, gelatin, and skim milk powder should be thoroughly mixed with a sterile spatula and a portion placed in a sterile sample bottle.

Frozen ice cream should be sampled with a sterile butter trier, after removing the surface inch from the point to be sampled. The core thus removed should be cut into short pieces with a sterile spoon or spatula and placed in a sterile bottle.

Care of the samples

All samples should be placed in cracked ice immediately and carried directly to the laboratory as quickly as possible.

Measuring the sample

The sample of ice cream may be either weighed or measured. With ice cream it is frequently difficult to expel all the air, with the result that one 10 cc. volume may contain more or less ice cream than another. Variations in the viscosity of the mix and in the cleanliness of the pipette may cause more or less of the mix to adhere to the glass. The gravimetric method has the disadvantage of being more tedious and more time-consuming, but, in general, gives more accurate and less variable results. The preference for the gravimetric or volumetric method will depend on the nature of the analytical work. For ordinary plant control work, where routine check is being made on the quality of the ingredients and the final product, the volumetric method is sufficiently accurate. On the other hand, for research and public health work, where accuracy and comparability of results are essential, the gravimetric method should be used. However, if consecutive samples are being taken at the various stages in the process of ice cream manufacture, all determinations should be made on the same basis, either gravimetric or volumetric. The former method gives results showing the estimated numbers of colonies developing on agar plates per gram, the latter, the number of colonies per cubic centimeter.

If the sample to be analyzed is frozen ice cream, it should be melted and the air expelled by heating in a water bath at 45°C. (113°F.) for fifteen minutes. If a higher temperature is used for melting the ice cream, it is likely to injure the organisms, and lower temperature is likely to necessitate a sufficiently long exposure to permit noticeable growth of the bacteria. When the volumetric method is used for ice cream, extreme care should be taken to insure complete expulsion of the air, and to rinse the pipette several times in the dilution water until the pipette is reasonably clean and free from ice cream.

Volumetric method. Liquids. The larger the volume of sample used in making the first dilution the less will be the per cent of error in measuring the sample. This is especially true of liquids of high viscosity such as heavy cream and melted ice cream mix.

The amounts most commonly used are: 1 cc. of sample in 99 cc. of sterile water, 5 cc. of sample in 95 cc. of sterile water, or 10 cc. of sample in 90 cc. of sterile water, giving dilutions of 1:100, 1:20, and 1:10 respectively. For all volumetric samples, the volume of the sample plus the volume of the sterile dilution water should equal 100 cc. The dilution, therefore, should be expressed as in "1:10," "1:100," etc., and the results in terms of "plate count per cubic centimeter—50,000, etc." Not more than two significant left-hand digits should be used in reporting numbers.

Gravimetric method. Solids. The results of gravimetric analyses are reported in terms of "plate count per gram—50,000 etc." Analyses of gelatin, sugar and skim milk powder, are usually made on a gravimetric basis, by weighing ten grams on sterile paper and placing in 90 cc. of sterile water under aseptic conditions. From this 1:10 dilution, appropriate dilutions are made and plated in the usual manner. In the analysis of gelatin it is necessary to warm the dilution blanks to insure equal distribution of the sample.

Fruits, such as strawberries in heavy syrup, may be weighed on sterilized paper conterpoised on the balances. Place two sheets of sterile glazed filter paper on the pans of the balance and weigh a 10 gram sample. Under aseptic conditions, introduce the fruit, together with the single thickness of filter paper, into the dilution bottle. In this manner the contamination of the paper from the scale pan is prevented.

Gravimetric method. Liquids. If a 10-gram sample of cream, melted ice cream, condensed milk, etc., is weighed directly into a dilution blank, it does not matter how much adheres to the pipette or how much air remains in the melted ice cream.

Method of weighing sample directly into dilution blank. A dilution blank containing 90 cc. of sterile water, and a pair of Cornet forceps are counter-balanced on a good grade of torsion balances. It is more convenient to have a beaker of water on each pan of the balance to facilitate counter-balancing. The instrument should be sufficiently sensitive so that one drop of water will disturb the balance. After counter-balancing to within one drop of the true balance, add a 10-gram weight to the pan op-

posite the dilution blank; remove the stopper from the dilution blank, taking care that it does not touch anything. Flame the mouth of the flask and, by means of a pipette introduce the sample to be analyzed until the 10-gram weight is accurately balanced. Flame the mouth of the dilution bottle and replace the stopper. Test the accuracy of the weighing by dropping one drop of the sample in the beaker on the opposite pan, if it is close enough so that one drop throws the balance to the other side, it may be considered sufficiently accurate for use in the analysis. If cotton stoppers are used in the dilution blanks, the Cornet forceps are used to suspend the stopper over the edge of the pan so that it is not contaminated by touching the pan. If glass- or rubber-stoppered dilution bottles are used, the Cornet forceps are unnecessary.

PREPARATION OF AGAR PLATES

Media. For ice cream analysis, several media have been found satisfactory, and others not mentioned here, no doubt, could be used with equal satisfaction. Most public health laboratories, however, use the standard agar as described in detail in the fifth edition of Standard Methods of Milk Analysis, published by the American Public Health Association, 370 Seventh Avenue, New York City. Briefly, this media is prepared as follows: Add 0.3 per cent Bacto-beef-extract (or other brands giving comparable results), 0.5 per cent Bacto-peptone (or other brands giving comparable results), and 1.5 per cent market agar to the desired number of liters of distilled water; dissolve in an autoclave for from forty to ninety minutes after the pressure has reached 15 pounds, the length of time depending on the quantity of media being prepared. Remove the media and determine its reaction. If necessary, standardize the reaction to pH 6.6. This may be done according to the method described in the Standard Methods of Milk Analysis. After standardization, the media should be filtered through cotton or Buchner funnels. Place 10 cc. of media in test tubes or 55 cc. in flasks and sterilize at 15 pounds pressure for twenty minutes or by heating in flowing steam on three successive days for twenty minutes each day.

Milk powder agar, as described by Ayers and Mudge, has been found very advantageous in the determination of different types of bacteria in ice cream. The following specific directions for making milk powder agar are quoted from the original article by Ayers and Mudge.[2]

Detailed directions for preparation of 1 liter of milk powder agar

Medium A ingredients for 1 liter:

(a) { 5 grams skim milk powder
1 gram sodium dibasic phosphate (Sörensen's phosphate) ($Na_2HPO_4-2H_2O$) } In 250 cc. distilled water

(b) { 5 grams peptone
3 grams extract } In 250 cc. distilled water

Mix (a) and (b) and add 500 cc. of double strength (3 per cent) washed-agar solution.

The detailed directions must be followed accurately if satisfactory and constant results are to be obtained. The medium is very easy to prepare when the various steps are understood and the process completed once. It may appear complicated because of the complete details which are given of each step in the process and which make the preparation of the medium appear somewhat long.

To make the milk-powder solution "a" use a good grade of skim-milk powder made by the spray process, and prepare the following solutions:

(1) { 5 grams milk powder
20 cc. distilled water

(2) { 1 gram sodium dibasic phosphate (Sörensen's phosphate)
5 cc. distilled water

(When making more than 1 liter, the same proportions of milk powder to water and phosphate to water must be used; therefore, to make 5 liters multiply each amount by 5.)

Weigh out 5 grams of skim-milk powder and pour on to 20 cc. of cold distilled water in a small beaker. Stir until thoroughly dissolved. In another beaker, dissolve 1 gram of sodium dibasic phosphate (Sörensen's phosphate) in 5 cc. of distilled water. Warm to dissolve phosphate quickly. Sörensen's phosphate, $Na_2HPO_4-2H_2O$, must be used.

[2] Ayers, S. H., and C. S. Mudge. Milk powder agar for the determination of bacteria in milk. Jour. Bacteriol., 5: 565–588 (1920).

Add the phosphate solution (2) to the milk powder (1). Place the beaker, containing (1) and (2) mixed, in a water bath with water at about 30°C. and heat the milk powder phosphate solution to about 60°C. This should take about ten minutes. At temperatures between 50° and 60°C. a flocculent grayish precipitate will appear. Continue the heating until the precipitate appears, then steam in an Arnold or other steamer for five minutes, or until the precipitate appears white. Then dilute the milk-powder solution about one-third with distilled water and steam five minutes longer. Too long steaming will cause the solution to turn dark and should be avoided. The whole heating period should not be more than twenty or twenty-five minutes.

Decant the solution while hot on to a filter paper, taking care to keep the precipitate in the beaker until most of the liquid is through. Then pour the precipitate on the filter and wash with a little distilled water. If the milk-powder solution has been properly heated it will filter readily, provided the filter paper is not too hard. "J. Green" Grade 588 and "Ilmenau" filter paper have given good results.

The filtrate, which is of a yellowish-white color, will appear cloudy and can not be filtered clear in the concentration used. This makes no difference, because it is clear in the dilution of the final medium.

Make up the filtered milk-powder solution to 250 cc. with distilled water. This completes the milk-powder solution "a."

To make the peptone-extract solution "b" dissolve 5 grams peptone and 3 grams Liebig extract in 100 cc. distilled water by steaming in the Arnold sterilizer or by boiling over flame for twenty minutes. Filter until clear and make solution up to 250 cc. with distilled water. This completes solution "b." "Difco" peptone has been used in our experiments because of its hydrogen-ion concentration, which is near the neutral point, and because, with the extract in the proportion of 5 grams peptone to 3 grams extract, a precipitate is usually formed which permits filtration with a resulting clear solution. For a standard medium, whatever makes of peptone and extract are selected should be universally used.

The milk-powder solution "a" is now mixed with the peptone extract solution "b" which gives a total volume of 500 cc. To this mixture 500 cc. of double strength (3 per cent) washed-agar solution is added. This completes the medium, which is now ready for sterilization.

We specifically mention distilled water. Tap water may or may not contain dissolved substances, the effect of which on media making and bacterial growth is unknown. By the use of distilled water this un-

certainty is obviated and one is assured of a constant definite solvent, "standard" everywhere.

A stock solution of double strength (3 per cent) washed agar is prepared and put up in flasks and sterilized. This agar is then ready at any time for use. The agar should be put up in flasks in amounts suitable for the amount of medium to be made at any one time. It is not desirable to use part of a flask of agar, then resterilize and hold for future use. Repeated heating lowers its jelly strength. The flasks should be stoppered to prevent evaporation. To prepare a liter of 3 per cent washed agar, weigh out 30 grams of agar and place in a flask with 200 cc. of distilled water. This proportion should always be used. Allow it to stand for twenty-four hours, at room temperature, with occasional shaking. Then pour off as much water as possible, using a piece of cheesecloth over the top of the flask, and add distilled water enough to make up again the original volume.

Allow the agar to stand another twenty-four hours, and then pour off the agar on to a cotton flannel cloth in a funnel and wash once with a liter of distilled water. Let the agar drain and then press out as much water as possible by squeezing the filter cloth with the hands. A container large enough to hold the agar is counterpoised on the laboratory scales, and the agar placed in it. In the opposite pan is placed 30 grams for the agar and 1000 grams for the weight of the water in which the agar is to be dissolved. Then water enough is added to make up this weight. This will make a liter of 3 per cent agar. Dissolve the agar by heating in the Arnold sterilizer, then filter through cotton flannel or absorbent cotton until clear.

Whey agar and casein digest agar have not been so widely used in ice cream analysis. Carbohydrate media such as dextrose agar, lactose agar, etc., may be prepared by adding one per cent of the carbohydrate to the plain agar previously described. The addition of carbohydrate to the plain medium increases the number of colonies appearing on the plates.

If it is desired to check the results of analysis in one laboratory with those obtained in another laboratory, it is essential that the media employed in both laboratories be the same.

Making the agar plates

After placing 1 cc. of the desired dilutions in Petri dishes, 10 cc. of agar should be admitted to each plate under aseptic conditions.

If the agar is in flasks, care should be taken to pour the same amount of agar into each plate. The number of plates poured will depend upon the accuracy desired. Ordinarily, duplicate plates should be prepared from each dilution, although this may not be necessary in routine plant control work.

Incubation

The Standard Methods of Milk Analysis recommends incubation of plates for forty-eight hours at 37°C. A more complete count will be obtained by adopting the plan followed in some laboratories of incubating forty-eight hours at room temperature, followed by forty-eight hours at 37°C.; still other laboratories use one week incubation at room temperature. If results are to be compared with the results of another laboratory, the incubation time and temperature should be the same in both laboratories.

Plates should be inverted during incubation to prevent spreaders. The atmosphere in the incubator should be kept moist so as to prevent the drying up of the media. Care should always be taken not to stack plates in piles of more than three, in order to insure equal incubation of all plates. Plates should not be disturbed during incubation, otherwise the water of condensation will flow across the plate and cause growth on the surface of the agar.

Counting the colonies

A convenient method of counting the colonies is to invert the plate and mark each colony with ink or a wax pencil as it is counted, at the same time keeping count with a tally meter in the other hand. This method greatly increases the accuracy of counting by preventing confusion of the count, due to interruptions or to counting the same colony twice. All plates in a series should be counted at as nearly the same time as possible. After counting the colonies with the naked eye, all plates should be carefully examined with a hand lens ($3\frac{1}{2}\times$) to detect colonies too small to be seen with the naked eye. Occasionally, it may prove necessary to examine the plates under the low power of a com-

pound microscope in order to determine the true nature of apparent colonies.

MICROSCOPIC COUNT OF BACTERIA IN ICE CREAM (FABIAN'S MODIFICATION OF THE BREED METHOD)[3]

In working with ice cream it is often desirable to obtain certain information that cannot be obtained by any other method than that of examining a small amount of ice cream directly under the microscope. While there has not been as much work done with this method for ice cream as has been done with milk, yet the committee feels that there is sufficient merit in the method to incorporate it in this report. The direct microscopic method for ice cream may be used to best advantage with the unpasteurized product and in the raw materials going into the mix. However, it is also of service in detecting the presence of excessive numbers of bacteria, use of moldy fruits and the like in the pasteurized product.

Apparatus required

The apparatus required for this method is the same as that used in the Breed method as applied to milk, excepting an analytical balance. A microscope, microscopic slide, an analytical balance, guide plate (2 by 4½ inches), staining jars, stain, xylol, alcohol, etc., should be available.

Preparation of ice cream film

The ice cream film, to be representative, should be made from a thoroughly mixed sample. Slides used in this work should be exceptionally clean if the best results are to be obtained. The slides are placed on the balance and counterpoised; 0.01 gram of ice cream is then weighed upon each end of the slide as quickly as possible in order to reduce the error due to evaporation. A platinum loop has been found to work very well for putting the ice cream on the slide. The 0.01 gram of ice cream is then spread uniformly over an area of 1 square centimeter, using a clean needle.

[3] Fabian, F. W. A bacteriological study of the homogenization process in making ice cream. Jour. Dairy Sci., 8: 246–269 (1925).

This area may be conveniently measured by placing the slide upon the guide plate or any surface ruled in square centimeter areas. After the film is spread, the slide should be placed upon a warm surface for drying. The drying should not be too rapid for this may crack the film and cause it to peel from the slide. However, the drying should be completed quickly (within ten minutes) or growth may take place.

After the film has been prepared as above described, the slides are ready for the staining process. They are first dipped in xylol or other suitable fat solvent for five minutes to remove the fat. Longer exposure is necessary for ice cream than for milk, due to the larger amount of fat present. The xylol is then drained off and the slide again placed on a warm surface to evaporate the remainder of the xylol. The slides are next immersed in 70 to 95 per cent grain or denatured alcohol for about twenty minutes. The alcohol is poured off and the slide again placed on a warm surface. The film is then stained thirty seconds with Loeffler's methylene blue, prepared as follows: saturated alcoholic solution of methylene blue, 30 cc., caustic potash (0.01 per cent solution), 100 cc. Care should be taken in selecting the methylene blue. Fresh preparations of the stain from a satisfactory source should be used.

The film is then ready to be decolorized. This may be done by first draining off the excess stain, and placing the slide in 70 to 95 per cent alcohol. The length of time it should remain in the alcohol is a matter of judgment and experience; usually dipping the slide in and out once or twice is sufficient. In case the decolorization is carried too far, the preparation may be re-stained without apparent injury. The film should be warmed again after it has been decolorized; this seems to be necessary for best results.

In routine practice staining jars may be used to good advantage. Place four jars in a row, containing xylol, 95 per cent alcohol, Loeffler's methylene blue, and 95 per cent alcohol, respectively. The preparation of four slides may be started and carried through the whole procedure at the same time, and these followed by four more, thus saving considerable time.

Standardizing the microscope

In order to obtain quantitative data the size of the microscopic field must be known. The only equipment necessary for standardization of the microscopic field is a stage micrometer ruled in hundredths of a millimeter, and a suitable ocular (6.4× gives approximately the field desired). A mechanical stage is not necessary but very convenient for this work.

The diameter of the microscopic field should be adjusted to 0.205 mm., by focusing the microscope on the graduations of the stage micrometer, and adjusting the draw tube so that the desired diameter is obtained. Each field will then cover an area of $\frac{1}{8028}$ square centimeter ($\frac{1}{8000}$ square centimeter is the approximate figure and the one generally used). When this adjustment is made, then about $\frac{1}{300,000}$ part of a gram of the dried ice cream is visible in one microscopic field. The number of bacteria found in each field, therefore, should be multiplied by 300,000 to give the estimated number of bacteria per gram. Obviously, more than a single field should be counted, and the average number per field used in the calculation. For routine work at least twenty-five representative fields should be counted for each sample of ice cream.

A special ocular micrometer with a circular ruling divided into quadrants should be used for counting ice cream containing few bacteria. With this micrometer, adjust the microscope so that the diameter of the circle on the eye-piece is 0.146 mm. This changes the value of each field examined to $\frac{1}{600,000}$ part of a gram, and the number of bacteria found in each field should be multiplied by 600,000 to give the estimated number of bacteria per gram. This method has the added advantage of examining only the center of the field where definition is good.

Significance of the microscopic count

No bacterial standard, based on this method, has been worked out for ice cream. However, it is felt that the method gives certain data as to the type of bacteria present that can be obtained best in this way.

This method, like all known methods for determining numbers of bacteria, should be regarded as giving an estimate only. Errors due to evaporation during weighing, inaccuracies in weighing such small amounts of ice cream, faulty staining, uneven spreading of the ice cream, counting objects that resemble bacteria, or failing to see bacteria that are present, are all possible sources of error. While these limitations are recognized as important, yet it is felt that they may be sufficiently overcome to make this method a valuable adjunct in determining the quality of unpasteurized ice cream.

The microscopic preparations of ice cream are more pitted than with milk, due to the large per cent of fat present.

Where the ice cream mix has been pasteurized, the presence of large numbers of dead bacteria makes the microscopic count unreliable for determining the quality of ice cream. However, it is especially advantageous for certain types of work, such as studying the breaking up of bacterial clumps after homogenizing or freezing.

METHODS OF CONTROLLING THE SANITARY QUALITY OF ICE CREAM

Methods of sterilization[4] *of the utensils and equipment*

The sterilization of utensils and equipment is just as necessary in an ice cream plant as in a market milk plant, a creamery or a condensery. In general, sterilization should be accomplished with heat rather than with chemicals. After thorough washing, the utensils should be treated with steam or hot water. Drying is necessary if bacterial growth is to be prevented and this may be accomplished with driers or by such thorough heating with steam or hot water that the moisture remaining after careful draining will be evaporated.

All piping should be of the sanitary type and should be taken down and thoroughly washed, steamed or scalded, and dried each time it is used. The vats employed for mixing or holding should be given just as careful attention as the small containers. Ho-

[4] The term sterilization, as used in this case, refers to practical sterility and not absolute sterility.

mogenizers and similar machines are very difficult to keep in a sanitary condition and may be important sources of contamination unless considerable attention is given to them. These machines should be thoroughly washed so that there will be as much drying as possible. Before using, it may be advisable to run hot water through the machine for several minutes in an attempt to remove or destroy the organisms that may have developed in the machine since it was cleaned. Surface coolers need careful attention and preferably should be steamed or scalded just before using.

Freezers present a difficult problem in sterilizing with steam because the low temperature at which they are operated necessitates slow and gradual warming before the steam is admitted. The freezer should either be allowed to stand an hour after washing and before using hot water and steam, or rinsed three or four times with gradually increasing temperatures of the successive rinse waters. If the temperature is gradually increased in this manner, the machine will not be injured by the introduction of steam.

Cans, vats, freezers, etc., which are to be dried by the heat in the metal, should be so left that the water vapor can readily escape. When the drying is complete such equipment may be closed to prevent air contamination, although this source of contamination is not usually serious in a plant where there is considerable moisture about.

The use of chemical disinfectants

Chloramine and hypochlorite disinfectants are frequently used in ice cream plants, especially in the sterilization of freezers. In some plants large volumes of disinfectant are pumped through the entire system. This gives satisfactory results, especially if used in conjunction with steam. Intelligent use of chemical disinfectants necessitates extreme care in the preparation and use of fresh solutions, in order to insure a high available chlorine content.

A home made sodium hypochlorite disinfectant may be made according to the following directions. Add enough warm water to 10 pounds of fresh chloride of lime (bleaching powder) to make

a moist paste. After this is done add the remainder of 3 gallons of warm water (not over 120°F). Break up all the lumps in the lime and strain the solution through screen wire. Dissolve 16 pounds of washing soda (sodium carbonate) in 3 gallons of warm water, and add this solution to the chloride of lime solution in a 10-gallon stone jar (do not use metal container). Allow the mixture to stand about twelve hours, stirring two or three times at intervals. Draw off the clear supernatant liquid into gallon earthenware jugs, keeping the liquid as clear as possible and free from the insoluble residue. Discard the residue. The stock disinfectant solution thus obtained will keep a long time if the jugs are tightly stoppered.

For disinfecting freezers, pipe lines, pumps, cans, and other equipment, use a dilution of 1 part stock solution to 100 parts of water. Make a new dilution of the disinfectant each day. It is important that all equipment should be thoroughly cleaned and brushed before the disinfectant is used. Allow the disinfectant to act about three minutes to secure the best results. Where steam is used in addition to the disinfectant, use the disinfectant before using the steam.

Importance of quality of ingredients

The bacteriological condition of certain of the ingredients[5] employed has a direct relationship to the bacterial content of ice cream, both when they are pasteurized and when they are not. In general, the dairy products used in ice cream are the most important sources of bacteria. The quality of the cream and milk used in the mix is a very important consideration in controlling the number of bacteria in the mix.

All dairy products used in the mix should be as fresh as it is possible to obtain them. Both natural and re-constituted cream vary a great deal in bacterial content even after they are heated. Bulk condensed milk may be an important source of organisms, especially when it is held without refrigeration, as may be the case during transportation.

[5] Olson, N. E., and A. C. Fay. The bacterial content of ice cream. Jour. Dairy Sci., 8: 415–444 (1925).

Gelatin varies a great deal in bacterial content, although there apparently has been a big decrease during recent years in the number of organisms present in this product, due presumably to improved methods of manufacture. In many cases the heating incident to dissolving the gelatin destroys a great majority of the organisms so that the contamination from gelatin may be much less than the original bacterial content of the product would indicate. The small amount of gelatin ordinarily used also tends to make this product responsible for only a small per cent of the organisms in the finished ice cream. In some instances, however, a high bacterial content is accompanied by an objectionable odor and poor gelatinizing properties.

Sugar which is properly protected from dust and dirt ordinarily contains only small numbers of organisms, and is usually insignificant as a source of bacteria in ice cream.

Vanilla extract usually contains only a small number of bacteria, due, in part at least, to the alcohol content.

Importance of plant sanitation

Sanitary conditions are just as desirable in plants in which ice cream is manufactured as in factories handling other food products. Floors, walls, and ceilings which are easily cleaned and impervious to water, an abundance of sunlight, good air conditions, etc., are important considerations when a plant is being built. In the operation of the plant, advantage should be taken of those factors and the plant kept in a clean condition. While proper sterilization of utensils and equipment are undoubtedly more important from the standpoint of the bacterial content of the ice cream than the condition of the plant, a clean, well lighted and well ventilated establishment suggests careful methods to the employees, and establishes confidence among the consumers.

Importance of cleanliness of operators

Cleanliness of the operators is an important factor in the handling of all food products. If the operators are careless in their personal habits, in the cleaning of equipment, etc., an influence

on the bacterial content of the ice cream is to be expected. Most diseases of human beings are of human origin, so that personal contact in the manufacture of any food should always be avoided whenever possible. Dirty clothing may be a source from which dust and dirt get into the ice cream, and, moreover, suggests carelessness to both the operator and visitors. The possibility of pathogenic organisms coming from the mouth, nose, hands, etc., makes careful habits among the employees very necessary.

Routine samples which should be taken to discover the cause of high bacterial counts

High bacterial counts from ice cream indicate that something is wrong, either with the quality of the raw materials or with the methods of manufacture, and, accordingly, the immediate cause should be located at once. The bacterial content of the various raw materials used, especially those which are likely to undergo bacterial changes, should be determined. These should be examined in the condition in which they are used and not in the condition in which they are received. The bacterial content of the mix before pasteurization should be determined first, and if this is found to be satisfactory, the analysis of the individual raw material may not be necessary. If the mix is held or aged, counts should be made after storage as well as before. Serious contamination from a piece of equipment can be detected by examining the material before and after it passes through this equipment. With certain machines, for example the homogenizer, the first material through is likely to be much more seriously contaminated than the material going through later. The tendency of such machines as the homogenizer and the freezer to cause an apparent increase in the bacterial content, due to the breaking up of the clumps of bacteria, must be taken into account in studying their influence on the bacterial count.

It is frequently advisable to take samples of the finished product just before it leaves the plant, and also after it has been held in the retailer's cabinet. Alternate thawing and freezing of ice cream, due to inefficient refrigeration should always be avoided.

Interpretative value of bacterial numbers

The total numbers of bacteria in dairy products have been used as an index to the sanitary quality for many years. The use of this index is based on the assumption that the conditions which favor the admission and growth of bacteria are not in keeping with the commonly accepted ideas of the manner in which dairy products should be handled. The factors which cause large numbers of bacteria in ice cream are, poor quality of ingredients, inefficient pasteurization, insanitary equipment, improper aging conditions, and sloven, careless methods of the employees. That is to say, ice cream with a high bacterial content has been neglected in one or more of the above factors. It is possible, however, that ice cream with a low bacterial content may have been made from poor quality ingredients, and that fact masked by pasteurizing at a high temperature. The most serious difficulty with the index value of bacterial numbers is the unavoidable high per cent of error in the count in any method of estimation yet devised. This fact should always be borne in mind in using the results as indexes to insanitary conditions. If used by inspectors or officials the results should only be interpreted as a guide to the plants needing attention and not as an infallible index of insanitary conditions.

To the plant operator, high bacterial counts should indicate that there is something wrong with the methods, or the quality of the ingredients. If detailed inspection of the plant does not reveal to him the cause of high counts, it will be necessary to take routine samples of the manufacturing process to locate the trouble.

High bacterial counts should not be construed by the inspector to mean that the plant is insanitary or that the product is necessarily unsafe, but rather that there is something faulty about the plant methods which can be revealed only by more detailed inspection. On the other hand, ice cream having a low bacterial count should not be regarded as above reproach from a sanitary point of view, due to the possibility of masking the poor quality of ingredients by the use of high temperature or long time of

pasteurization. It is always advisable to combine inspection with laboratory findings in order to draw conclusions from the results.

Excessive numbers of bacteria in ice cream are significant largely through the faulty methods which their presence reveals, and not because excessive numbers of bacteria indicate the presence of dangerous disease germs. At best, the bacterial count can be used only as an aid to the inspector and not as a substitute for inspection.

The views expressed in this report are the views of a committee appointed by the American Dairy Science Association. As such they are printed for the general criticism of other members of the A. D. S. A., and any other interested parties.

Separate copies of this report may be secured at cost from the Chairman of the Committee on Bacteriological Methods.

Sub-Committee on Bacteriological Methods of Examining Ice Cream:

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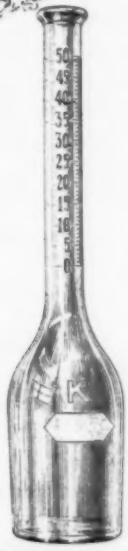

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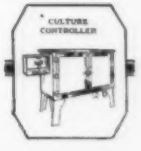

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